FEB 16 1988

504G12

HE20.3159/2:320

NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 320

TOXICOLOGY AND CARCINOGENESIS STUDIES OF ROTENONE

(CAS NO. 83-79-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

COMPLETED

ORIGINAL

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is made up of four charter DHHS agencies: the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF ROTENONE

(CAS NO. 83-79-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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January 1988

NTP TR 320

NIH Publication No. 88-2576

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NOTE TO THE READER

This study was performed under the direction of the National Institute of Environmental Health Sciences as a function of the National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for public peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

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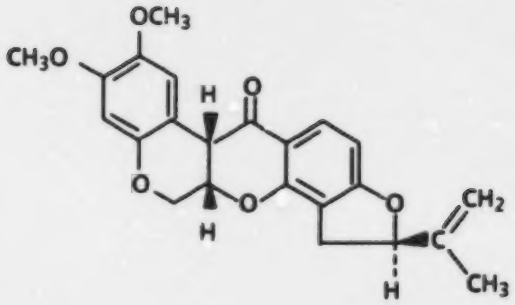

ROTENONE

CAS No. 83-79-4

1,2,12,12,a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)-[1]benzopyrano[3,4-b]furo[2,3-h][1]benzopyran-6(6H)-one

$C_{23}H_{22}O_6$ Molecular weight 394.4

Trade names of formulations: Derrin, Derris, Tubatoxin, Nicouline, Prentox, Noxfish, Rotocide, Barbasco, Cube Root, Haiari, Dactinol

ABSTRACT

Toxicology and carcinogenesis studies of rotenone (more than 98% pure), a pesticide, were conducted in B6C3F$_1$ mice and F344/N rats for 14 days, 13 weeks, and 2 years.

Results of the Fourteen-Day Studies: **In 14-day studies (dietary rotenone concentrations of 0-600 ppm in the first 14-day studies and 0-4,800 ppm in the second 14-day studies), rough hair coats and dose-related decreases in mean body weight gain were observed in rats. Rats fed diets containing rotenone at concentrations of 1,200 ppm or higher lost weight. No compound-related toxic effects were observed in mice.**

Results of the Thirteen-Week Studies: **In 13-week studies (concentrations of 0-1,200 ppm rotenone in feed for rats and 0-50,000 ppm for mice), compound-related effects included lower body weight gain in rats at 150 ppm or more and in mice at 5,000 ppm or more; deaths in rats at 600 ppm or more and in mice at 1,600 ppm or more; and bone marrow atrophy and inflammation and hyperplasia of the forestomach in male rats at 300 ppm or more and in female rats at 150 ppm or more. These findings were used to establish the dietary concentrations of rotenone for the 2-year studies.**

Experimental Design for the Two-Year Studies: **Two-year studies of rotenone were conducted by administering diets containing 0, 38, or 75 ppm rotenone to groups of 50 F344/N rats of each sex for 103 weeks. Groups of 50 B6C3F$_1$ mice of each sex were administered diets containing 0, 600, or 1,200 ppm rotenone on the same schedule. The estimated average amount of rotenone consumed per day was 1.7 mg/kg or 3.5 mg/kg for low dose or high dose rats and 115 mg/kg or 250 mg/kg for low dose and high dose mice.**

Survival and Mean Body Weight in the Two-Year Studies: **Survival of control and dosed rats was similar (male: control, 22/50; low dose, 31/50; high dose, 30/50; female: control, 27/50; low dose,**

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32/50; high dose, 31/50). Mean body weights of dosed and control male rats were comparable. Mean body weights of high dose female rats were 5%-9% lower than those of the controls between weeks 58 and 88. Survival of high dose male mice was significantly greater than that of the controls (male: 29/50; 36/50; 47/50; female: 37/50; 42/50; 45/50). Final mean body weights of dosed mice were lower than those of the controls by 8%-13% for males and 17%-24% for females.

Neoplastic Effects in the Two-Year Studies: Parathyroid gland adenomas were observed in 1/41 control, 0/44 low dose, and 4/44 high dose male rats. The historical incidence of this uncommon tumor in untreated control male rats in NTP studies is 4/1,314 (0.3%). Because these tumors are rare and because the highest incidence ever seen in a control group is 1/50, the increase in these tumors may have been related to rotenone administration.

The incidence of subcutaneous tissue fibromas, fibrosarcomas, sarcomas, myxosarcomas, or neurofibrosarcomas (combined) in low dose female rats was greater ($P<0.05$) than that in the controls (0/50; 5/50; 3/50). These tumors were combined because of their possible common histiogenic origin from fibroblasts or undifferentiated mesenchymal cells. The incidence of those tumors in the low dose females was greater than the historical rate at this laboratory (9/337, 3% ± 1%) and throughout the Program (50/2,021, 2% ± 2%). Because of the lack of a significant dose-related trend and because statistical significance was attained only by combining tumors of differing morphology, the subcutaneous tissue tumors in female rats were not considered to be chemically related. The incidences of these tumors in dosed male rats were not significantly different from that in the controls.

Hepatocellular adenomas or carcinomas (combined) occurred in male mice with a negative ($P<0.02$) trend, and the incidence in the high dose group was lower than that in the controls (12/47; 12/49; 1/30). Because this low rate of combined liver tumors is unusual, this decrease may have been related to rotenone administration.

Subcutaneous tissue fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) in male mice occurred with a significant ($P<0.05$) negative trend (8/49; 4/50; 2/50). The incidence in the high dose group was significantly lower than that in the controls by the life table test ($P=0.01$).

Genotoxicity: Rotenone was not mutagenic when tested according to a preincubation protocol with *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with or without metabolic activation by rat or hamster liver S9. Rotenone induced forward mutations in the mouse L5178Y/TK$^{+/-}$ lymphoma assay without activation; it was not tested in the presence of S9. Results of tests with rotenone in Chinese hamster ovary cells were negative for induction of sister chromatid exchanges (SCEs) in the absence of exogenous metabolic activation (at concentrations at which the chemical was very toxic), equivocal for SCEs in the presence of rat liver S9 (due to a nonrepeatable positive response when tests were conducted up to toxic concentrations), and negative for chromosomal aberrations in both the presence and absence of metabolic activation.

Data Audit: An audit of the experimental data was conducted for the 2-year studies of rotenone. No data discrepancies were found that influenced the final interpretations.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of rotenone for male F344/N rats, as indicated by an increased incidence of parathyroid gland adenomas (uncommon tumors). There was *no evidence of carcinogenic activity* in female F344/N rats fed diets containing 38 or 75 ppm rotenone. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice fed diets containing 600 or 1,200 ppm rotenone for 2 years. The decreased incidence of liver neoplasms in male mice may have been related to the administration of rotenone.

SUMMARY OF THE TWO-YEAR FEED AND GENETIC TOXICOLOGY STUDIES OF ROTENONE

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Dietary Concentration			
0, 38, or 75 ppm rotenone	0, 38, or 75 ppm rotenone	0, 600, or 1,200 ppm rotenone	0, 600, or 1,200 ppm rotenone
Body weights in the 2-year study			
Dosed and controls similar	Dosed lower than controls	Dosed lower than controls	Dosed lower than controls
Survival rates in the 2-year study			
22/50; 31/50; 30/50	27/50; 32/50; 31/50	29/50; 36/50; 47/50	37/50; 42/50; 45/50
Neoplastic effects			
Parathyroid adenomas	None	None	None
Level of evidence of carcinogenic activity			
Equivocal	No evidence	No evidence	No evidence
Genetic toxicology (a)			

	Salmonella	Mouse L5178Y/TK$^{+/-}$	CHO cells in vitro	
Responses:	(gene mutation)	(gene mutation)	SCE	Aberration
(−S9/+S9)	−/−	+/not tested	−/equivocal	−/−

(a) + = positive, − = negative; S9 is liver enzyme fraction used for exogenous metabolic activation.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 10-11.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans.

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

These considerations together with the definitions as written should be used as composite guidelines for selecting one of the five categories. Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the induction by chemicals of more neoplasms than are generally found, or the earlier induction by chemicals of neoplasms that are commonly observed. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on rotenone on August 19, 1986, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF ROTENONE

On August 19, 1986, the draft Technical Report on the toxicology and carcinogenesis studies of rotenone received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. K. Abdo, NTP, introduced the studies by reviewing the experimental design, results, and proposed conclusions (equivocal evidence of carcinogenic activity for male and female rats; no evidence of carcinogenic activity for male or female mice).

Dr. Scala read the review from Dr. Hughes, a principal reviewer, who could not attend. Dr. Hughes agreed with the conclusions as written for male and female mice. For male rats, he suggested that the increased incidence of parathyroid gland adenomas in high dose rats may have been due to sampling error, and he felt that the absence of increases in the incidence of parathyroid gland hyperplasia also mitigated against rotenone influence. Dr. S. Eustis, NIEHS, said that the tissue accountability of parathyroid glands from control, low dose, and high dose animals was 41/50, 44/50, and 44/50, respectively, which shows even sampling across all groups. Dr. J. Huff, NIEHS, mentioned that these numbers of sections were quite good, given the small size of the parathyroid glands. Dr. Purchase argued that unless step sectioning is used, sampling error with such a tiny organ could still be a factor. Dr. Eustis thought that the potential problem was being overemphasized, in that similar numbers of sections were evaluated for each group and that serial sectioning would not influence the overall proportions of neoplasia. For female rats, Dr. Hughes suggested that the lack of a dose response and the atypical zero incidence of subcutaneous tumors in controls helped mitigate against a rotenone effect.

As a second principal reviewer, Dr. Sivak agreed with the conclusions for mice but thought that the conclusions for male and female rats should be lowered to no evidence of carcinogenic activity. He noted the low incidence of microscopic tumors in the parathyroid gland in males and the need to pool subcutaneous tumors from different areas to attain statistical significance in females, along with the inverted dose response and the longer time to tumor in the high dose groups than in controls. Dr. Sivak requested that the rationale for dose selection in mice be expanded and felt that more discussion should be given about the large species differences in the biologic response to the chemical. Dr. Abdo said that the species differences in sensitivity represented large biologic variations that could not be explained from the available data, although metabolism studies might be useful and the discussion would be expanded.

As a third principal reviewer, Dr. Chinchilli agreed with the conclusions as written for both rats and mice. Since the only tumors with increased incidences in rats occur rarely, he said that it would be helpful if the NTP could provide statistical tests incorporating historical control data from the same laboratory to aid in evaluating the significance of rare tumors. Dr. J. Haseman, NIEHS, responded that the NTP generally does not perform statistical analyses against historical control data because there is no consensus as to which statistical technique is most appropriate and, even more importantly, because there are unresolved uncertainties regarding the comparability of tumor diagnoses and reported incidences across studies.

In other discussion with regard to the decreased incidence of liver neoplasms in male mice, Dr. Huff pointed out that this was the first time the Program has stated that a decreased incidence of a tumor was associated with chemical administration. Dr. Purchase noted that the Program, in evaluating the significance of increased tumor incidence, considers a finding of increased incidence of the same

tumor in the other sex or the other species to be supportive. Conversely, he thought that the significant negative trend for subcutaneous tumors in male mice should weaken the rationale for associating the lesions in female rats with chemical administration. Dr. Huff responded that correlations from one sex of one species to the other sex of another species were somewhat more difficult.

Dr. Sivak moved that the Technical Report on rotenone be accepted with the conclusions as written for mice, no evidence of carcinogenic activity, but with the conclusions for rats changed to no evidence of carcinogenic activity. Dr. Purchase seconded the motion. Dr. Mirer made an alternative motion to consider male and female rats separately. As Chair, Dr. Scala indicated that better progress could be made by separate motions. The motion that the conclusion for male mice be accepted was approved unanimously by 10 votes. The motion that the conclusion for female mice be accepted was approved unanimously by 10 votes. Dr. Mirer's procedural motion to consider male and female rats separately was seconded and approved by nine votes to one (Dr. Popp). Dr. Sivak restated his motion of no evidence of carcinogenic activity for male rats based on possible sampling error and on a low control tumor incidence; the motion was defeated by five votes to four (Dr. Capen, Dr. Crowley, Dr. Purchase, and Dr. Sivak), with one abstention (Dr. Mirer). Dr. Sivak restated the motion of no evidence of carcinogenic activity for female rats, and it was approved by six votes to four (Dr. Chinchilli, Dr. Hooper, Dr. Perera, and Dr. Popp). To complete the evaluation for male rats, Dr. Hooper moved that the conclusion for male rats, equivocal evidence of carcinogenic activity, be accepted as written. Dr. Gallo seconded the motion. Dr. Purchase offered an amendment that there be a reexamination of the parathyroid glands to assess whether there was a sampling error. Dr. Eustis said that trying to obtain additional sections would be extremely difficult. Dr. Popp agreed and said that step sections would yield an incomplete answer because part of the tissue was already gone due to previous sectioning. The amendment was defeated by eight votes to two (Dr. Purchase and Dr. Sivak). Dr. Hooper's motion to accept the conclusions as written for male rats resulted in a tie vote, with four reviewers agreeing (Dr. Chinchilli, Dr. Hooper, Dr. Perera, and Dr. Popp), four disagreeing (Dr. Capen, Dr. Crowley, Dr. Purchase, and Dr. Sivak), and two abstaining (Dr. Gallo and Dr. Mirer). Dr. Scala, as Chair, voted in favor of the motion to break the tie.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Rotenone is based on the 13-week studies that began in July 1980 and ended in October 1980 and on the 2-year studies that began in June 1981 and ended in June 1983 at Battelle Columbus Laboratories.

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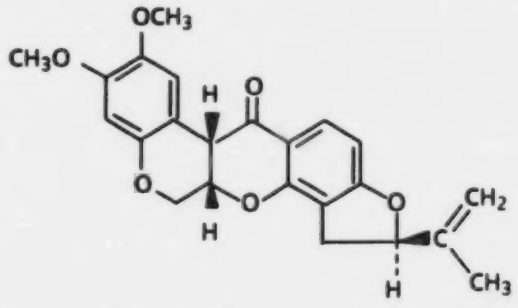
I. INTRODUCTION

Physical and Chemical Properties
Production, Formulations, and Use
Biochemical Effects
Metabolism
Pharmacologic Effects
Toxicity
Carcinogenicity
Teratogenic and Reproductive Effects
Genotoxicity
Study Rationale

I. INTRODUCTION



ROTENONE

CAS No. 83-79-4

1,2,12,12,a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl)-
[1]benzopyrano[3,4-b]furo[2,3-h][1]benzopyran-6(6H)-one

$C_{23}H_{22}O_6$ Molecular weight 394.4

Trade names of formulations: Derrin, Derris, Tubatoxin, Nicouline, Prentox, Noxfish, Rotocide, Barbasco, Cube Root, Haiari, Dactinol

Rotenone is a natural insecticidal and piscicidal constituent of several plant species belonging to the Leguminoseae family. Most commercial rotenone preparations come from the roots of *Derris elliptica*, *D. mallancensis*, *Lonchocarpus utilis*, and *L. urucu*. Preparations from Derris sp. are called derris or tuba; those from Lonchocarpus sp. are called timbo (Hayes, 1982). The pure compound is obtained by extraction from the roots of these plants with carbon tetrachloride, acetone, or benzene and purification by crystallization (Farm Chem. Handbook, 1982). Rotenone is synthesized by reacting a mixture of morpholine enamine or pyrrolidine enamine with tubaic acid chloride or by condensing pyrrolidine enamine and dicyclohexylcarbodiimide with tubaic acid to give dehydrorotenone, which is then reduced to rotenone (Miyano, 1965). The insecticidal and piscicidal activity of rotenone is attributed to its ability to inhibit electron transfer in the mitochondrial respiratory chain (Oberg, 1961).

Physical and Chemical Properties

Rotenone is a solid and forms orthorhombic crystals in trichloroethylene. It is practically insoluble in water and soluble in alcohol, acetone, carbon tetrachloride, chloroform, or ether. Solutions of rotenone exposed to light and air are readily oxidized; colorless solutions turn successively yellow, light orange, and finally deep red. Crystalline deposits that contain dehydrorotenone and rotenone may appear in these oxidized solutions (Merck Index, 1983). Some physical properties of rotenone are listed in Table 1.

TABLE 1. PHYSICAL PROPERTIES OF ROTENONE

Density	1.27 g/ml at 20° C
Melting point Dimorphic form	165°-166° C 185°-186° C
Optical rotation	$[\alpha]_D$ at 20° C is −228°

Production, Formulations, and Use

In the United States, 100,000 pounds (45,000 kg) of rotenone was used in 1978. In 1982, the United States imported approximately 690,000 pounds (314,000 kg) of rotenone and rotenone-containing roots (Chem. Econ. Handbook, 1984). Rotenone is available as a technical-grade solution at concentrations of 35%, 90%, or 95%, as a

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formulation intermediate at a concentration of 50%, and as a wettable powder at concentrations of 5% or 20%. It is also available as a 5% emulsifiable concentrate (USEPA, 1980).

Rotenone is a nonsystemic insecticide used on fruit trees, such as apple, apricot, peach, persimmon, pomegranate, and quince, to control aphids, maggots, bagworms, codling moths, Japanese beetles, and leaf hoppers. It is widely used on vegetable plants to control Mexican bean beetles, cabbage worms, aphids, thrips, stinkbugs, flea beetles, and vegetable weevils. It is also used to control grubs, ticks, lice, and fleas on cattle. Rotenone has been used on humans for external treatment of chiggers (2% lotion) and scabies (10% emulsion) (NIOSH, 1983).

Emulsifiable concentrates and wettable powders of rotenone are used in lakes, ponds, and reservoirs to control undesirable fish such as bullheads, carp, freshwater catfish, golden shiner, green sunfish, sucker, and bony fish (USEPA, 1980).

Biochemical Effects

Rotenone inhibits respiration by blocking the oxidation of the reduced nicotinamide adenine dinucleotide (NADH) (Lindahl and Oberg, 1961; Ernster et al., 1963). This effect may be overcome by the addition of vitamin K_3 (Ernster et al., 1963). Oxidation of choline by rat liver mitochondria was inhibited by rotenone, and this inhibition could be abolished by acetate ions (Feinberg et al., 1967). The incorporation of acetate into squalene and cholesterol in human placental and rat liver tissue preparations was inhibited by rotenone (Boguslawski and Zelewski, 1971). Rotenone increased incorporation of radiolabeled acetate into long-chain fatty acids by rabbit heart mitochondria in the presence of citrate, isocitrate, malate, or succinate ions. This effect may be due to the ability of rotenone to block the oxidation of NADH and thus maintain a high NADH to NAD^+ ratio (Hull and Whereat, 1967).

Metabolism

Rotenone is metabolized by the liver mixed-function oxidase system of mammals. The biotransformation reactions include hydroxylation at carbons 6 and 24 to give rotenolone I and rotenolone II, hydroxylation at the terminal methyl group in the isopropenyl side chain to give 8'-hydroxyrotenone, and epoxidation of the double bond in the isopropenyl side chain followed by hydrolysis to give 6',7'-dihydro-6,7'-dihydroxyrotenone (Yamamoto and Casida, 1967). Natural rotenone (5'-B rotenone) was found to undergo 3-O demethylation in mice (Unai et al., 1973). After radiolabeled rotenone was administered orally to rats and mice, 20% of the radioactivity was recovered in the urine (Fukami et al., 1969).

Pharmacologic Effects

Rotenone produces an anesthetic effect when applied to mucous membranes or nerve axons (Hayes, 1982). At a concentration of 1-10 ppb, this compound inhibited the tonic response of intestinal smooth muscle of guinea pig ileum or rabbit duodenum to acetylcholine and histamine without preventing the immediate short-lasting contraction. A vasodilator effect was observed in dogs injected intra-arterially with 15-20 µg rotenone (Santi et al., 1963). Rotenone ($0.33\text{-}1.7 \times 10^{-5}$ M) dilated the vessels in perfused rabbit ear. A respiratory stimulant effect and a short-lasting hypotensive effect were observed in rabbits and dogs injected intravenously with 15-25 µg rotenone (Santi et al., 1966).

Toxicity

Toxic symptoms in humans include dermatitis in the genital region, ulcerative rhinitis with anosmia, and irritation of the lips and tongue in workers exposed to fine powders of derris. Ingestion of 0.5 g of timbo extract after eating caused depression and vomiting, but there were no disturbances when the stomach was empty. Acute rotenone poisoning causes numbness, nausea, vomiting, and tremors (Hayes, 1982).

The acute toxicity of rotenone has been reported for several mammalian species. The oral LD_{50} value was 60 mg/kg for rats and 1.5 mg/kg for guinea pigs. The oral lethal dose for an adult human male was estimated to be about 200 mg/kg. A dose of 2 g/kg did not kill dogs (Santi and Toth, 1965; Ambrose and Haag, 1937). A

dose of 3.7 mg/kg was fatal to pigs and caused salivation, vomiting, incoordination, and respiratory depression before death. Paralytic symptoms were observed in another instance of fatal poisoning in pigs (Oliver and Roe, 1957). The reported intravenous and intraperitoneal LD_{50} values in rats are 0.2 and 1.6 mg/kg (Santi and Toth, 1965).

Three of five dogs administered a daily oral dose of 10 mg rotenone died before the end of a 102-day study (Haag, 1933). The dogs that died had body weight loss and fatty metamorphosis of the liver and adrenal gland. The liver was also found to be the organ primarily affected in rats fed diets containing up to 200 ppm rotenone for 107 days (Ambrose et al., 1942). No adverse effects were noted in two male and two female beagle dogs fed diets containing 50, 100, or 400 ppm cube for 28 months (Hansen et al., 1965).

Carcinogenicity

Cube root powder (rotenone, 5.8%; other extractables, 12%; inert ingredients, 82.2%) was given at levels of 0, 50, 100, 250, 500, or 1,000 ppm in the diet for 2 years to groups of 24 or 25 Osborne-Mendel male rats and 25 or 26 female rats (Hansen et al., 1965). The powder at concentrations greater than 100 ppm retarded the growth of both sexes of the rats. Dosed female rats had enlarged livers and spleens; no differences in organ weight were noted between control and dosed male rats. No histologic changes attributable to cube root were noted.

Tubatoxin (chemically identical to rotenone and 90% pure) was not considered carcinogenic to (C57BL/6 × AKR)F_1 and (C57BL/6 × C3H/Anf)F_1 strains of mice (Innes et al., 1969). In this study, 18 mice of each sex and strain were given 1 mg tubatoxin/kg body weight per day in 0.5% gelatin by gavage from days 7 to 28 of age. For the following 18 months, the mice were fed the compound in the diet at a concentration of 3 ppm. Small numbers of animals, low doses, and short duration of exposure were used in these studies.

Female albino rats (strain unspecified) receiving daily intraperitoneal doses of 1.7 µg rotenone/kg body weight (in 0.1 ml sunflower oil) for 42 days had a 100% incidence of mammary tumors as compared with 0% in the controls (Gosalvez and Merchan, 1973). The tumors appeared 6-11 months after the end of dosing. Daily doses of 1.7 or 3 mg/kg given to 25 male and 25 female Sprague Dawley rats (intraperitoneally) or Wistar rats (by gavage) for 42 days did not produce mammary neoplasms after an additional 17 months of observation (Freudenthal et al., 1981). However, a slight increase in the incidences of fibrosarcomas and fibromas of the skin occurred in males in both the intraperitoneal and gavage studies and in the adrenal gland of males in the gavage study. The difference in incidences of mammary tumors in the aforementioned studies was attributed to a difference in the riboflavin content of the diets: the diets used by Gosalvez and Merchan were deficient in riboflavin whereas those used by Freudenthal et al. were enriched with this vitamin (Gosalvez, 1983).

Rotenone was not carcinogenic to weanling female Wistar rats (72 per group) receiving 0, 2.5, or 5.0 µmol/kg in sunflower oil solutions containing 10% chloroform (Allaben et al., 1984). The mice received intraperitoneal injections 5 days per week for 8 weeks and were observed for an additional 16 months. Rotenone at concentrations up to 1,000 ppm in the diet for 18 months was not carcinogenic to Syrian golden hamsters (Leber and Persing, 1979). Hamsters receiving 1,000 ppm rotenone in the diet had a final mean body weight 15% lower than that of controls.

Teratogenic and Reproductive Effects

The administration of 5 or 10 mg/kg technical-grade rotenone in corn oil by gavage to Wistar rats on days 6-15 of pregnancy reduced maternal body weight gain, fetal weight, and skeletal ossification and produced increased incidences of extra ribs (Khera et al., 1982). At a dose of 10 mg/kg per day, this compound was associated with increased infertility and resorptions. No significant effects were found at 2.5 mg/kg per day.

Decidualized pseudo-pregnant Sprague Dawley rats fed diets containing 10-1,000 ppm rotenone from days 6 to 10 of gestation had reduced body and uterine weights as compared with controls. Lethargy, ataxia, and rough, unkempt fur were

observed in rats at the 750- and 1,000-ppm doses. Similar effects were observed in pregnant rats fed diets containing 600 or 800 ppm rotenone on days 6-15 of gestation. No resorption of implantation sites was seen in rats receiving 10, 100, 200, 400, 600, or 800 ppm, and no abortions occurred (Spencer and Sing, 1982). The fetal survival rate was reduced at all doses.

Genotoxicity

Numerous gene mutation tests that used various strains of *Salmonella typhimurium* and *Escherichia coli*, with or without exogenous metabolic activation, have uniformly demonstrated that rotenone is not mutagenic in prokaryotes (Ashwood-Smith et al., 1972; Ficsor and LoPiccolo, 1972; Probst and Hill, 1980; Probst et al., 1981; Shirasu et al., 1981; Moriya et al., 1983). Results of NTP-sponsored testing support these negative results in bacteria. Rotenone, at concentrations up to 10,000 µg/plate, did not induce reverse mutations in *S. typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without liver S9 from Aroclor 1254-induced male Sprague Dawley rats or Syrian hamsters, by a preincubation protocol (Appendix E, Table E1). In the absence of metabolic activation, rotenone induced increases in forward mutations at the $TK^{+/-}$ locus of mouse L5178Y lymphoma cells at concentrations of 0.5-4.0 µg/ml in two out of three experimental trials; the chemical was not tested in the presence of S9 (Table E2).

Rotenone has been studied extensively for its ability to disrupt mitosis through a dual process of inhibition of spindle microtubule assembly and depletion of cellular ATP pools, which results in insufficient energy for specific mitotic requirements (Barham and Brinkley, 1976a,b; Meisner and Sorensen, 1966; De Brabander et al., 1976). In further investigations of compounds capable of disrupting oxidative phosphorylation, Hilton and Walker (1977) demonstrated that mouse L1210 leukemia cells in culture exposed for 60 minutes to 10^{-7} M rotenone exhibited extensive DNA damage (measured as irreversible strand separation in alkali) and a significant decrease in cellular ATP levels. The authors hypothesized that DNA damage after rotenone exposure in this system is a result of the reduction of cellular ATP levels, rather than a direct physical interaction of rotenone with cellular DNA. They focused on cellular endonucleases as the cause of DNA single strand breaks in these ATP-depleted cells.

An 8-hour exposure at concentrations up to 1,000 µM rotenone did not promote unscheduled DNA synthesis (UDS) in SV-40 transformed human fibroblast cells in culture with or without rat liver S9 (Ahmed et al., 1977). Also, adult rat hepatocytes did not exhibit UDS after exposure to rotenone in vitro (Probst and Hill, 1980; Probst et al., 1981).

Rotenone did not induce sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells when administered at concentrations at which the proportion of second division cells is reduced from nearly 100% (the control value) to 50% after a 24-hour culture period (Tomkins et al., 1980). In NTP-sponsored studies with CHO cells, results of tests for SCEs were also negative in the absence of metabolic activation. Rotenone was very toxic as indicated by toxicity at doses above 0.003 µg/ml and the necessity of delaying harvest by approximately 8 hours to provide suitable numbers of second-division metaphase cells. Results of tests for induction of SCEs in the presence of metabolic activation with S9 from the liver of Aroclor 1254-induced male Sprague Dawley rats were considered equivocal due to a nonreproducible positive response observed at near toxic doses (2-6 µg/ml) (Table E3). Results of tests for induction of chromosomal aberrations in CHO cells were negative both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 (Table E4).

Study Rationale

Rotenone was nominated for toxicology and carcinogenesis studies by the National Cancer Institute because of extensive human exposure through its use as a pesticide, because of the inadequacy of previous studies in which injection was the route of exposure, and because of conflicting data in the literature regarding its carcinogenicity. Rotenone was administered in the diet because rotenone is stable in feed and dietary administration is the most practical route of exposure.

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II. MATERIALS AND METHODS

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PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

FIRST FOURTEEN-DAY STUDIES

SECOND FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF ROTENONE

Rotenone was obtained in a single lot (lot no. 735-RAP-1502) from S.B. Penick and Company (Lyndhurst, New Jersey). The material in this lot was a white, microcrystalline powder with a melting point of 163°-165° C. The infrared, ultraviolet, and nuclear magnetic resonance spectra were consistent with those found in the literature (Figures 1 and 2).

Values for carbon and hydrogen obtained by elemental analysis agreed with theoretical values. Karl Fischer titration indicated a 0.07% water content. Thin-layer chromatography indicated a major spot and three trace impurities by each of two systems (carbon tetrachloride:ethyl acetate, 80:20 and hexanes:acetone, 70:30). High-performance liquid chromatography (HPLC) with a μBondapak C_{18} column and detection at 313 nm indicated a major peak and five impurities by system 1 (water:methanol, 40:60) and a major peak and four impurities by system 2 (water:methanol, 32:68). The total area of the impurity peaks was 1.3% of the major peak area for system 1 and 1.0% for system 2. Two of the impurities had retention times that matched literature values for the degradation products dehydrorotenone and rotenonone. The overall purity of this lot was estimated as greater than 98%.

No degradation was observed after 2 weeks' storage at temperatures up to 60° C. Periodic reanalysis of the bulk chemical by infrared spectroscopy and HPLC (μBondapak C_{18} column, methanol:water 70:30 or 60:40, mobile phase, 313-nm detection) indicated no notable degradation during the studies.

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

Formulated diets were made by preparing a rotenone/feed premix by hand which was then blended with plain feed in a Patterson-Kelly® blender for 15 minutes (Table 2). Homogeneity of feed blends containing 500 ppm rotenone was demonstrated to be within 1.5% of the target value from three locations in the blender. The formulated diets at concentrations of 500 ppm and 38 ppm were shown to be stable for at least 2 weeks when stored at temperatures of up to 25° C (Kline et al., 1986).

TABLE 2. PREPARATION AND STORAGE OF FORMULATED DIETS IN THE FEED STUDIES OF ROTENONE

First Fourteen-Day Studies	Second Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation			
Weighed quantities of feed mixed with weighed portions of rotenone in a Patterson-Kelly® twin-shell V blender for 15 min	Same as first 14-d studies	Same as first 14-d studies	Weighed portions of rotenone and feed premixed in a jar. Premix combined with additional weighed portion of feed and mixed in a 16-qt Patterson-Kelly® twin-shell blender for 15 min with the intensifier bar on for the first 5 min
Maximum Storage Time			
2 wk	2 wk	2 wk	2 wk
Storage Conditions			
4° C	4° C	4° C	4° C

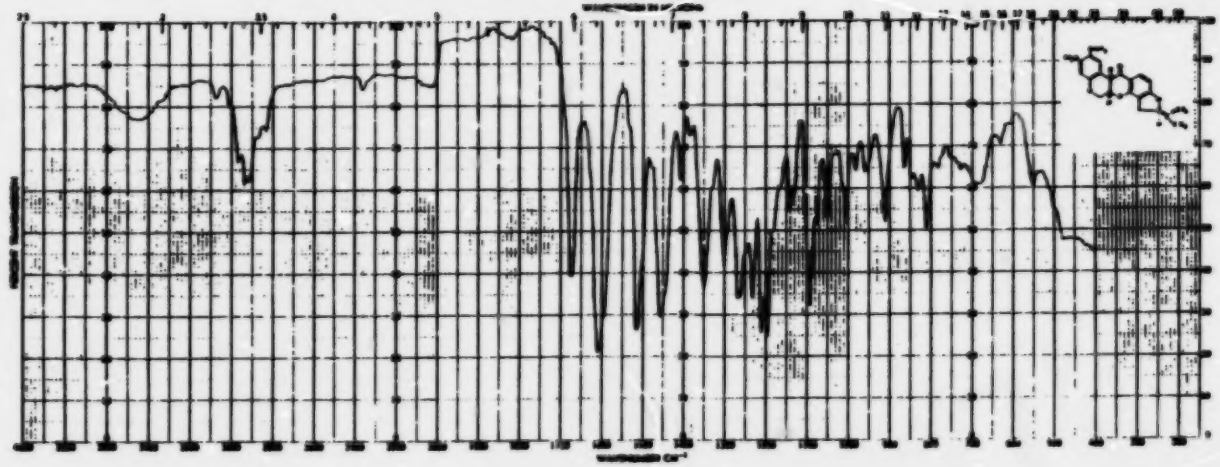

FIGURE 1. INFRARED ABSORPTION SPECTRUM OF ROTENONE (LOT NO. 735-RAP-1502)

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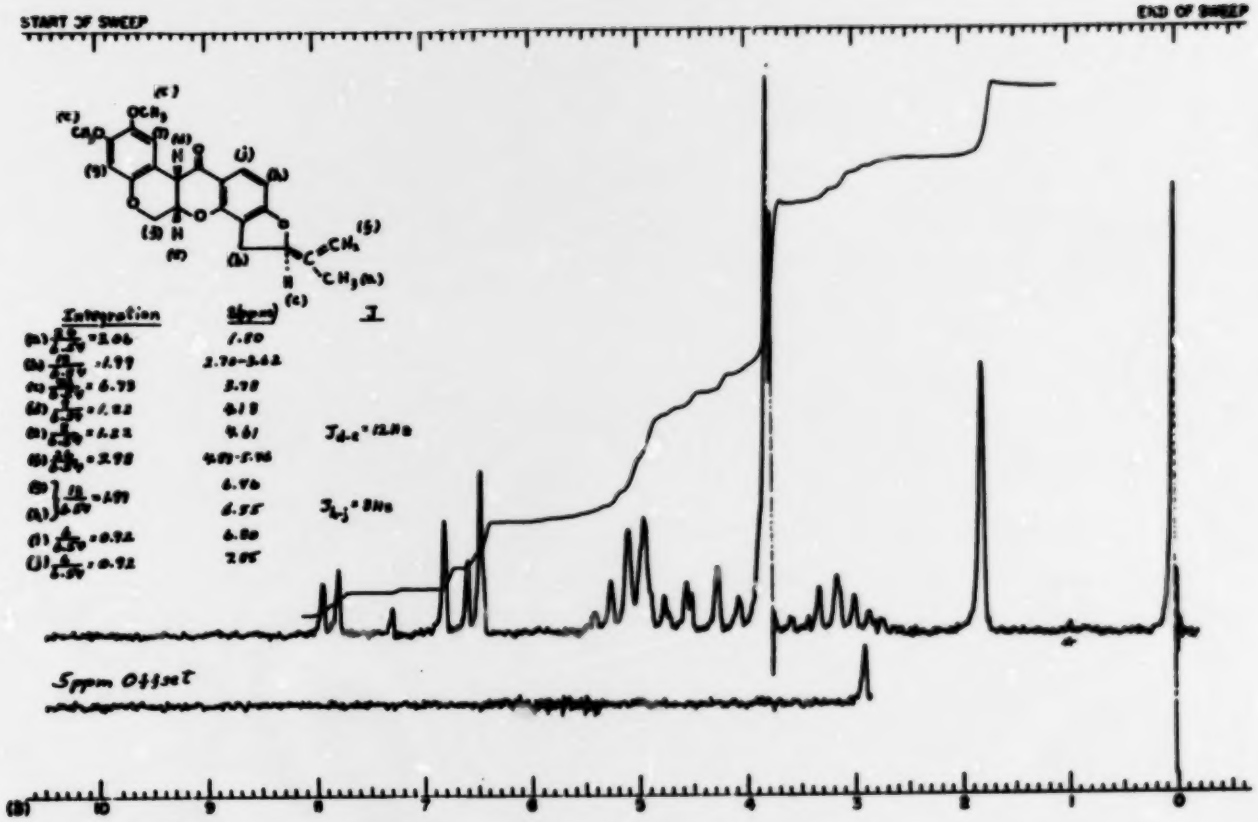

FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF ROTENONE (LOT NO. 735-RAP-1502)

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Periodic analyses of formulated diet mixtures of rotenone were conducted at the study laboratory and the analytical chemistry laboratory. Feed samples were extracted with acetonitrile:acetic acid (99:1) followed by HPLC analysis of the resultant extract with a μBondapak C_{18} column and detection at 294 nm. Formulated diets were analyzed twice during the 13-week studies. The results ranged from 108% to 93% of the target concentration (Table 3). During the 2-year studies, the formulated diets were analyzed at approximately 8-week intervals. Because 54/60 diet mixtures were formulated within ±10% of the target concentrations, it is estimated that diets were prepared within specifications approximately 90% of the time throughout the studies (Table 4). Of the six diet mixtures determined to be out of specifications, three were within ± 13% of target concentrations and three were 19%-26% above target concentrations. Periodic referee analyses by the analytical chemistry laboratory indicated generally good agreement between laboratories (Table 5).

TABLE 3. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE

Date Mixed	Concentration of Rotenone in Feed (ppm)		Determined as a Percent of Target
	Target	Determined (a)	
07/08/80	50,000 (bottom)	(b) 53,900	107.8
	50,000 (upper right)	(b) 47,400	94.8
	50,000 (upper left)	(b) 51,150	102.3
	16,000	16,100	100.6
	5,000	4,995	99.9
	1,900	2,050	107.9
	1,200	1,195	99.6
	600	580	96.7
	300	285	95.0
	150	154	102.7
	75 (bottom)	(b) 80	106.7
	75 (upper right)	(b) 82	109.3
	75 (upper left)	(b) 78	104.0
08/26/80	16,000	16,537	103.4
	5,000	5,403	108.1
	1,900	1,951	101.6
	1,200	1,190	99.2
	600	614	102.3
	600	569	94.8
	300	284	94.7
	150	142	94.7
	75	70	93.3

(a) Results of duplicate analysis
(b) Samples taken from different positions in the feed blender

TABLE 4. RESULTS OF ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF ROTENONE

Date Mixed	Determined Concentration of Rotenone in Feed for Target Concentration (ppm) (a)			
	38	75	600	1,200
06/01/81	41.4	71.4	--	--
06/09/81	--	--	631.0	1,197.0
03/20/81	38.9	77.4	608.5	(b) 1,510.0
08/26/81	--	--	(c) 615.4	(c) 1,179.5
10/06/81	34.7	76.0	630.6	(d) 1,442.4
10/15/81	--	--	--	(c) 1,256.3
11/04/81	--	--	--	(e) 1,234.5 (upper right)
	--	--	--	(e) 1,229.0 (upper left)
	--	--	--	(e) 1,245.0 (bottom)
12/03/81	35.2	73.6	647.5	1,276.5
02/18/82	39.7	73.9	617.0	1,266
04/21/82	34.4	67.8	597.8	1,274.4
06/01/82	(b) 42.9	74.5	637.6	1,267.6
06/05/82	(c) 34.9	(f) 82.3	586.5	1,216.3
07/20/82	34.6	(b) 66.9	582.4	1,162.5
07/27/82	--	(c) 83.0	--	--
07/30/82	35.1	75.2	--	--
	(b) 42.4	--	--	--
08/03/82	(c) 37.4	--	--	--
09/21/82	37.7	72.4	588.0	1,225.0
09/28/82	34.5	70.3	--	--
11/09/82	34.5	80.0	619.6	1,207.9
01/10/83	38.3	71.0	640.0	1,310.6
03/02/83	(b) 45.3	70.5	644.8	1,293.8
03/04/83	(c) 37.1	--	--	--
05/03/83	39.8	72.3	630.5	1,146.7
Mean (ppm)	38.1	72.9	618.0	1,268.9
Standard deviation	3.55	3.49	22.28	96.95
Coefficient of variation (percent)	9.3	4.8	3.6	7.6
Range (ppm)	34.4-45.3	66.9-80.0	582.4-647.5	1,146.7-1,510.0
Number of samples	16	15	14	15

(a) Results of duplicate analysis
(b) Out of specifications; not used in the study.
(c) Remix, not included in the mean
(d) Out of specifications; used to dose mice for 2 days.
(e) Samples taken from different positions in the feed blender; mean of three values used in calculation of the overall mean.
(f) Not used in the study or included in the mean

TABLE 5. RESULTS OF REFEREE ANALYSIS OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF ROTENONE

Date Mixed	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory (a)	Referee Laboratory (b)
06/01/81	38	41.4	37.2
12/03/81	75	73.6	73.7
06/01/82	1,200	1,267.6	1,223
11/09/82	600	619.6	601
05/03/83	75	72.3	74.1

(a) Results of duplicate analysis
(b) Results of triplicate analysis

FIRST FOURTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories. Rats were 7 weeks old when placed on study, and mice were 8 weeks old. Groups of five males and five females were fed diets containing 0, 50, 100, 200, 400, or 600 ppm rotenone for 14 consecutive days. Rats and mice were observed twice per day and were weighed on days 0, 7, and 14. A necropsy was performed on all animals. A histologic examination was performed on animals in the control and 600-ppm groups. Details of animal maintenance are presented in Table 6.

SECOND FOURTEEN-DAY STUDIES

Male and female rats were obtained from Harlan Industries; male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories. Rats were 9 weeks old when placed on study, and mice were 8 weeks old. Groups of five males and five females were fed diets containing 0, 300, 600, 1,200, 2,400, or 4,800 ppm rotenone for 14 consecutive days. Rats and mice were observed twice per day and weighed on days 0, 7, and 15 (rats) or 14 (mice). A necropsy was performed on all animals. Details of animal maintenance are presented in Table 6.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to rotenone and to determine the concentrations to be used in the 2-year studies.

Four- to five-week-old male and female F344/N rats and 5- to 6-week-old male and female B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories, observed for 14 days, and distributed to weight classes. Animals were assigned to cages and then to groups according to tables of random numbers. Diets containing 0, 75, 150, 300, 600, or 1,200 ppm rotenone were fed to groups of 10 rats of each sex. Diets containing 0, 600, 1,900, 5,000, 16,000, or 50,000 ppm rotenone were fed to groups of 10 mice of each sex.

Animals were housed five per cage. Formulated diets, control diets, and water were available ad libitum. Animals were checked two times per day; moribund animals were killed. Feed consumption was measured weekly by cage. Individual animal weights were recorded weekly. At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Liver weight to body weight ratios were determined at necropsy. Tissues and groups examined are listed in Table 6.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 38, or 75 ppm rotenone were fed to groups of 50 male and 50 female rats for 103 weeks. Diets containing 0, 600, or 1,200 ppm rotenone were fed to groups of 50 male and 50 female mice on the same schedule.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Frederick Cancer Research Center under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the study laboratory at 5 weeks of age. The rats were quarantined at the study laboratory for 20 days and the mice for 19 days. Thereafter, a complete pathologic examination was performed on five animals of each sex and species to assess their health status. The rats were 57 days old and the mice were 55 days old when placed on study. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F).

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF ROTENONE

First Fourteen-Day Studies	Second Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN			
Size of Study Groups			
5 males and 5 females of each species	Same as first 14-d studies	10 males and 10 females of each species	50 males and 50 females of each species
Doses			
0, 50, 100, 200, 400, or 600 ppm rotenone in feed	0, 300, 600, 1,200, 2,400, or 4,800 ppm rotenone in feed	Rats--0, 75, 150, 300, 600, or 1,200 ppm rotenone in feed; mice--0, 600, 1,900, 5,000, 16,000, or 50,000 ppm rotenone in feed	Rats--0, 38, or 75 ppm rotenone in feed; mice--0, 600, or 1,200 ppm rotenone in feed
Date of First Dose			
7/24/79	3/2/80	7/11/80	Rats--6/10/81; mice--6/15/81
Date of Last Dose			
8/6/79	3/15/80	Rats--10/14/80-10/15/80; mice--10/15/80-10/16/80	Rats--5/31/83; mice--6/9/83
Duration of Dosing			
14 consecutive d	14 consecutive d	13 wk	103 wk
Type and Frequency of Observation			
Observed 2 × d; weighed by cage on d 0, 7, and 14; feed consumption measured 1 × wk	Observed 2 × d; weighed individually on d 0, 7, and 15 (rats) or 14 (mice); feed consumption measured 1 × wk	Same as first 14-d studies except weighed initially and 1 × wk thereafter	Observed 2 × d; weighed 1 × wk for 8 wk and 1 × mo thereafter; clinically examined 1 × d for 5 mo and then 1 × mo
Necropsy and Histologic Examination			
Necropsy performed on all animals; histologic exam performed on control and 600-ppm groups	Necropsy performed on all animals; histologic exam not performed	Necropsy performed on all animals; histologic exam performed on the following tissues of rats in the 300-, 600-, and 1,200-ppm groups and of mice in the 5,000-, 16,000-, and 50,000-ppm groups: adrenal glands, brain, colon, esophagus, femur, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testis or ovaries/uterus, salivary glands, small intestine, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. The following tissues also examined: bone marrow and stomach for 75-ppm female rats; bone marrow, liver, and stomach for 150-ppm male rats; bone marrow for 150-ppm female rats; liver for 500-ppm female mice; liver, spleen, and testis for 1,900-ppm male mice; liver for 1,900-ppm female mice	Necropsy performed on all animals; the following tissues examined histologically for control and high dose groups: adrenal glands, brain, colon, esophagus, eyes, femur including marrow, gallbladder (mice), gross lesions and tissue masses, heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testis or ovaries/uterus, salivary glands, skin, small intestine, spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Gross lesions, parathyroids, and thyroid gland examined for low dose male rats; gross lesions for low dose female rats; gross lesions, liver, and lung for low dose male mice; and gross lesions for female mice

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF ROTENONE (Continued)

First Fourteen-Day Studies	Second Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE			
Strain and Species			
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source			
Charles River Breeding Laboratories (Portage, MI)	Rats--Harlan Industries (Indianapolis, IN); mice--Charles River Breeding Laboratories (Kingston, NY)	Same as first 14-d studies	Frederick Cancer Research Center (Frederick, MD)
Study Laboratory			
Battelle Columbus Laboratories	Battelle Columbus Laboratories	Battelle Columbus Laboratories	Battelle Columbus Laboratories
Method of Animal Identification			
Toe mark	Toe mark	Toe clip	Toe mark and ear mark
Time Held Before Study			
Rats--18 d; mice--19 d	Rats--19 d; mice--18 d	14 d	Rats--20 d; mice--19 d
Age When Placed on Study			
Rats--7 wk; mice--8 wk	Rats--9 wk; mice--8 wk	Rats--6-7 wk; mice--7-8 wk	8 wk
Age When Killed			
Rats--9 wk; mice--10 wk	Rats--11 wk; mice--10 wk	Rats--21 wk; mice--22 wk	113 wk
Necropsy Dates			
8/7/79	Rats--3/17/80; mice--3/18/80	Rats--10/14/80-10/15/80; mice--10/15/80-10/16/80	Rats--6/6/83-6/8/83; mice--6/13/83-6/16/83
Method of Animal Distribution			
Animals assigned from weight classes to cages according to a table of random numbers and then to dose groups according to another table of random numbers	Same as first 14-d studies	Same as first 14-d studies	Same as first 14-d studies
Feed			
Purina Lab Chow® (Ralston Purina Co., St. Louis, MO); available ad libitum	NIH 07 Rat and Mouse Ration (Zeigler Bros., Gardners, PA); available ad libitum	Same as second 14-d studies	Same as second 14-d studies
Bedding			
Absorb-Dri (Lab Products, Garfield, NJ)	Same as first 14-d studies	Same as first 14-d studies	Same as first 14-d studies
Water			
Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as first 14-d studies	Same as first 14-d studies	Same as first 14-d studies
Cages			
Polycarbonate (Lab Products, Inc., Garfield, NJ)	Same as first 14-d studies	Same as first 14-d studies	Same as first 14-d studies
Cage Filters			
Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as first 14-d studies	Same as first 14-d studies	Same as first 14-d studies

TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE FEED STUDIES OF ROTENONE (Continued)

First Fourteen-Day Studies	Second Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)			
Animals per Cage			
5	5	5	5
Other Chemicals on Study in the Same Room			
None	None	None	None
Animal Room Environment			
Temp--21°-23° C; * hum--40%-60%; ** fluorescent light 12 h/d; 15 room air changes/h	Same as first 14-d studies	Same as first 14-d studies	Same as first 14-d studies

* Temperature was within the specified range 88% of the time; maximum recorded, 24° C; minimum recorded, 10° C.
** Relative humidity was within the specified range 92% of the time; maximum recorded, 76%; minimum recorded, 18%.

Animal Maintenance

Animals were housed five per cage. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 6.

Clinical Examinations and Pathology

All animals were observed two times per day. Clinical signs were recorded daily for 5 months and then once per month. Body weights by cage were recorded once per week for the first 8 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed or cannibalized, missexed, or found missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Histopathologic examination of tissues was performed according to the "inverse pyramid" design (McConnell, 1983a,b). Complete histopathologic examinations (Table 6) were performed on high dose and control animals and on all animals dying early in the studies, including those in lower dose groups. In addition, histopathologic examinations were performed on all gross lesions and tissues/organs from animals in the lower dose groups when chemically related neoplastic or nonneoplastic effects were identified in the high dose animals. If mortality in a high dose group exceeded that in the control group by 15%, complete histopathologic examinations were performed on all of the animals in the second highest dose group in addition to those in the high dose group.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues

and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG, which includes the laboratory pathologist, without knowledge of previously render diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided.

Life Table Analysis--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an

animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analysis--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, and week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of primary tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

III. RESULTS

RATS

FIRST FOURTEEN-DAY STUDIES

SECOND FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

FIRST FOURTEEN-DAY STUDIES

SECOND FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

FIRST FOURTEEN-DAY STUDIES

All the rats survived to the end of the studies (Table 7). The final mean body weights of dosed male rats were 8%-13% lower than that of the controls. The final mean body weights of female rats that received 200, 400, or 600 ppm were 4%, 8%, or 13% lower than that of the controls. Feed consumption by dosed groups was similar to that of the controls. No compound-related clinical signs or gross or microscopic pathologic effects were observed. Because rotenone was not toxic at the dietary concentrations tested, a second 14-day study at higher concentrations (up to 4,800 ppm) was conducted.

SECOND FOURTEEN-DAY STUDIES

Deaths occurred in males at 2,400 and 4,800 ppm and in females at 2,400 ppm (Table 8). Dose-related decreases in weight gain and feed consumption were observed. The rats at 1,200, 2,400, or 4,800 ppm lost weight. The weight loss ranged from 16% to 43% of initial body weight. Compound-related effects included rough hair coats in all males and females at 1,200, 2,400, and 4,800 ppm and hard feces and hunched posture in all males and females at 2,400 and 4,800 ppm. No compound-related gross pathologic effects were observed. Because there were no deaths or compound-related lesions in rats receiving rotenone at 1,200 ppm or less, the 1,200-ppm dietary concentration was selected as the highest concentration to be used in the 13-week studies.

TABLE 7. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE FIRST FOURTEEN-DAY FEED STUDIES OF ROTENONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams)			Final Weight Relative to Controls (percent)	Feed Consumption(c)	
		Initial	Final	Change (b)		Week 1	Week 2
MALE							
0	5/5	153	226	+73	--	18.7	19.5
50	5/5	151	207	+56	91.6	17.6	17.7
100	5/5	152	208	+56	92.0	17.2	17.5
200	5/5	157	213	+56	94.2	16.6	17.2
400	5/5	146	197	+51	87.2	16.0	16.6
600	5/5	150	203	+53	89.8	17.8	17.8
FEMALE							
0	5/5	139	158	+19	--	13.8	14.3
50	5/5	136	155	+19	98.1	13.1	13.4
100	5/5	137	156	+19	98.7	13.7	14.0
200	5/5	134	151	+17	95.6	14.3	13.3
400	5/5	138	146	+8	92.4	15.5	14.5
600	5/5	137	137	0	86.7	15.0	13.3

(a) Number surviving/number in group
(b) Mean weight change of the group
(c) Grams of feed consumed per animal per day

TABLE 8. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE SECOND FOURTEEN-DAY FEED STUDIES OF ROTENONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	130 ± 3	189 ± 7	+59 ± 4	--	25.2	25.2
300	5/5	128 ± 4	176 ± 5	+48 ± 3	93	21.6	23.4
600	5/5	131 ± 4	167 ± 8	+36 ± 4	88	21.6	23.3
1,200	5/5	128 ± 3	108 ± 9	-20 ± 7	57	16.2	16.3
2,400	(e) 2/5	131 ± 3	79 ± 2	-56 ± 3	42	11.1	11.3
4,800	(f) 4/5	131 ± 3	90 ± 10	-43 ± 7	48	12.2	11.9
FEMALE							
0	5/5	105 ± 1	138 ± 2	+33 ± 2	--	18.8	17.3
300	5/5	106 ± 4	134 ± 5	+28 ± 3	97	17.2	18.7
600	5/5	102 ± 4	102 ± 8	0 ± 6	74	16.7	16.5
1,200	5/5	105 ± 4	83 ± 6	-22 ± 5	60	16.0	16.3
2,400	(g) 1/5	103 ± 2	69	-36	50	12.0	11.7
4,800	5/5	105 ± 2	71 ± 3	-34 ± 3	51	11.8	11.8

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams of feed per animal per day
(e) Day of death: 12,13,14
(f) Day of death: 15
(g) Day of death: 9,12,13,15

THIRTEEN-WEEK STUDIES

All 10 males and 6/10 females that received 1,200 ppm and 3/10 males and 4/10 females that received 600 ppm died before the end of the studies (Table 9). The final mean body weights of male rats that received 300 or 600 ppm were 21% and 52% lower than that of the controls; females that received 150 or 300 ppm weighed 16% and 26% less than controls. Female rats that received 600 or 1,200 ppm lost weight. The liver weight to body weight ratios of male rats that received 300 ppm and of female rats that received 600 or 1,200 ppm were increased relative to that of the controls (Table 10). This finding may reflect primarily the reduced body weight in the dosed animals. Rough hair coats and arched backs were observed for males and females that received 1,200 ppm. Generalized weakness in males and rough hair coats and arched backs were observed in rats that received 600 ppm. The incidences and severity of bone marrow atrophy were generally dose related (Table 11). The incidence of forestomach lesions was dose related (Table 12).

Dose Selection Rationale: Based on weight gain depression, the incidence of deaths, and bone marrow depletion, doses selected for rats for the 2-year studies were 38 and 75 ppm rotenone in feed.

TABLE 9. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 12
MALE							
0	10/10	122 ± 4	357 ± 6	+235 ± 4	--	19.3	15.8
75	10/10	126 ± 4	344 ± 5	+218 ± 4	96	17.9	14.7
150	10/10	132 ± 2	340 ± 5	+208 ± 6	95	17.5	16.7
300	10/10	128 ± 3	282 ± 5	+154 ± 5	79	17.8	15.6
600	(e) 7/10	122 ± 4	170 ± 8	+51 ± 11	48	16.7	16.3
1,200	(f) 0/10	123 ± 3	(g)	(g)	--	18.5	--
FEMALE							
0	10/10	101 ± 3	203 ± 3	+102 ± 2	--	13.6	11.6
75	10/10	103 ± 3	192 ± 4	+89 ± 3	95	12.8	10.0
150	10/10	101 ± 2	170 ± 2	+69 ± 2	84	12.9	11.4
300	10/10	103 ± 3	150 ± 2	+47 ± 4	74	16.0	9.8
600	(h) 6/10	104 ± 3	93 ± 4	-7 ± 6	46	15.3	15.6
1,200	(i) 4/10	100 ± 3	84 ± 12	-17 ± 14	41	15.8	11.5

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams of feed per animal per day
(e) Week of death: 6,13,13
(f) Week of death: 3,3,4,4,5,6,6,6,6,6
(g) No data are reported due to the 100% mortality in the group.
(h) Week of death: all 13
(i) Week of death: 4,4,6,6,6,8

TABLE 10. LIVER WEIGHT TO BODY WEIGHT RATIOS OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE (a)

Concentration (ppm)	No. Examined	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/Necropsy Body Weight (mg/g)
MALE				
0	10	365 ± 20	13,850 ± 1,563	37.9 ± 3.88
75	(b) 10	359 ± 15	(c) 15,744 ± 1,439	44.2 ± 4.20
150	10	359 ± 19	14,234 ± 1,494	39.7 ± 3.99
300	10	(d) 299 ± 40	13,232 ± 1,305	(c) 45.0 ± 7.52
600	7	(d) 198 ± 15	(d) 8,780 ± 1,560	44.5 ± 8.57
FEMALE				
0	10	204 ± 12	7,382 ± 502	36.2 ± 3.46
75	10	206 ± 10	(d) 8,533 ± 839	41.5 ± 2.63
150	10	195 ± 7	7,262 ± 629	37.2 ± 2.38
300	10	179 ± 5	7,522 ± 668	42.2 ± 4.11
600	5	126 ± 8	7,540 ± 911	(d) 60.2 ± 8.55
1,200	4	110 ± 22	6,812 ± 589	(d) 64.0 ± 15.08

(a) Mean ± standard deviation; P values are versus the controls by Dunnett's test (Dunnett, 1955).
(b) One final body weight was not taken; data for body weight and ratio are for nine animals.
(c) $P<0.05$
(d) $P<0.01$

TABLE 11. INCIDENCE AND SEVERITY OF BONE MARROW ATROPHY IN RATS IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE (a)

	Concentration (ppm)					
	0	75	150	300	600	1,200
Male	0/10	--	0/10	10/10 (2.2)	10/10 (2.8)	9/10 (3.9)
Female	0/10	1/10 (1.0)	6/10 (1.0)	9/10 (1.8)	9/10 (2.2)	8/10 (3.8)

(a) Mean severity in animals with the lesion is in parentheses: 1, minimal; 2, mild; 3, moderate; 4, severe.

TABLE 12. INCIDENCE AND SEVERITY OF FORESTOMACH LESIONS IN RATS IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE (a)

	Concentration (ppm)				
	0	75	150	300	600
MALE					
Inflammation	0/10	--	0/10	3/10 (2.3)	4/8 (3.8)
Hyperplasia	0/10	--	0/10	3/10 (2.3)	4/8 (2.8)
FEMALE					
Inflammation	0/10	0/10	4/10 (2.3)	7/9 (2.3)	3/7 (3.0)
Hyperplasia	0/10	0/10	5/10 (2.8)	6/9 (2.5)	4/7 (2.0)

(a) Mean severity in animals with the lesion is in parentheses: 1, minimal; 2, mild; 3, moderate; 4, severe.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed and control male rats were comparable (Table 13 and Figure 3). Mean body weights of high dose female rats were 5%-9% lower than those of the controls between weeks 58 and 88. The average daily feed consumption by low dose and high dose rats was 101% and 102% that of the controls for males and 94% and 96% for females (Appendix G, Tables G1 and G2). The estimated average amount of rotenone consumed per day was 1.7 mg/kg or 3.4 mg/kg for low dose or high dose male rats and 1.8 mg/kg or 3.6 mg/kg for low dose or high dose female rats.

TABLE 13. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF ROTENONE

Weeks on Study	Control		38 ppm			75 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	204	50	199	98	50	197	97	50
1	220	50	214	97	50	214	97	50
2	246	50	242	98	50	243	99	50
3	261	50	252	99	50	258	99	50
4	277	50	272	98	50	273	99	50
5	289	50	286	99	50	287	99	50
6	305	50	299	98	50	300	98	50
7	315	50	309	98	50	311	99	50
8	326	50	321	99	50	322	99	50
13	348	50	347	100	50	350	101	50
17	386	50	379	98	50	382	99	50
22	408	50	398	98	50	405	100	50
26	412	50	414	100	50	416	101	50
30	427	50	426	100	50	430	101	50
35	440	50	442	100	50	443	100	50
40	454	50	454	100	50	451	99	50
44	459	50	460	100	50	456	99	50
49	461	50	464	101	50	460	100	50
54	469	49	465	100	50	459	99	50
58	461	49	469	102	49	465	101	49
63	464	49	465	100	49	458	99	49
68	465	48	466	100	49	460	99	49
70	464	47	465	100	49	459	99	49
75	471	46	467	99	48	461	98	48
79	464	46	473	102	47	460	99	47
83	472	41	467	99	46	463	98	45
88	472	37	467	99	42	464	98	41
93	466	33	457	98	41	467	100	38
97	456	28	454	100	37	457	100	35
101	448	22	446	100	33	448	100	33
103	432	22	440	102	31	439	102	30
FEMALE								
0	143	50	142	99	50	146	102	50
1	153	50	154	101	50	157	103	50
2	180	50	180	100	50	182	101	50
3	184	50	184	100	50	184	100	50
4	172	50	171	99	50	173	100	50
5	178	50	176	99	50	178	100	50
6	182	50	181	99	50	180	99	50
7	185	50	184	99	50	183	99	50
8	189	50	187	99	50	187	99	50
13	196	50	195	99	50	194	99	50
17	209	50	207	99	50	206	99	50
22	219	50	218	100	50	216	99	50
26	233	50	230	99	50	217	97	50
30	230	50	226	98	50	226	98	50
35	236	49	234	99	50	232	98	50
40	250	49	245	98	50	244	98	50
44	261	49	256	98	50	254	97	50
49	271	49	264	97	50	259	96	50
54	280	47	275	98	50	270	96	50
58	291	47	284	98	50	277	95	50
63	288	47	286	99	50	276	96	50
68	305	45	292	96	49	282	92	50
70	309	45	295	95	45	282	91	49
75	326	43	312	96	48	297	91	49
79	331	43	318	96	47	304	92	47
83	337	42	325	96	45	313	93	44
88	341	41	324	95	43	322	94	40
93	339	40	331	98	41	331	98	38
97	341	34	325	95	38	333	97	35
101	350	29	330	94	33	333	95	34
103	343	27	321	94	33	332	97	31

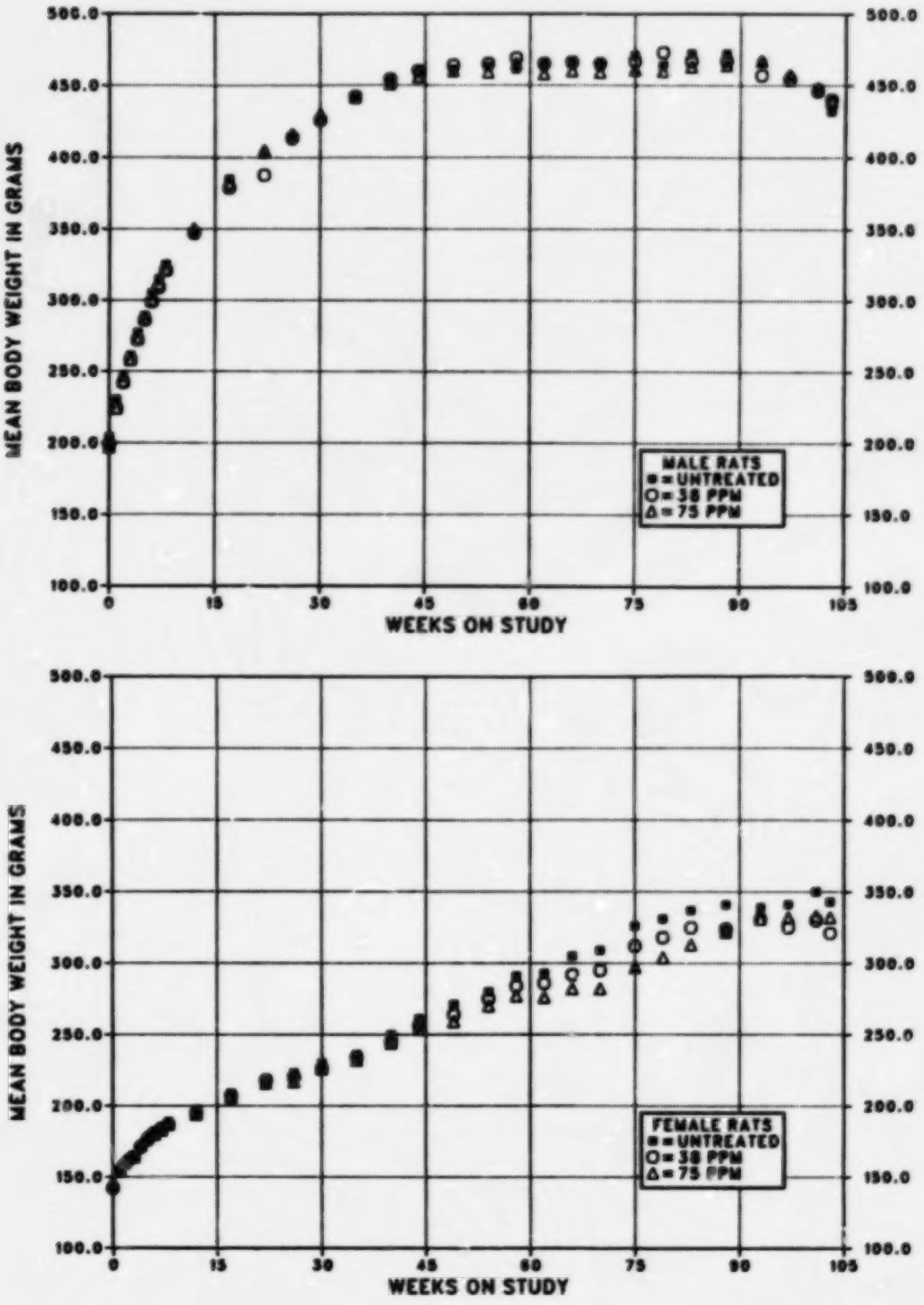

FIGURE 3. GROWTH CURVES FOR RATS FED DIETS CONTAINING ROTENONE FOR TWO YEARS

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Survival

Estimates of the probabilities of survival for male and female rats fed diets containing rotenone at the concentrations used in these studies and for controls are shown in Table 14 and in the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed for any group of either sex.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the subcutaneous tissue, parathyroid, and anterior pituitary gland.

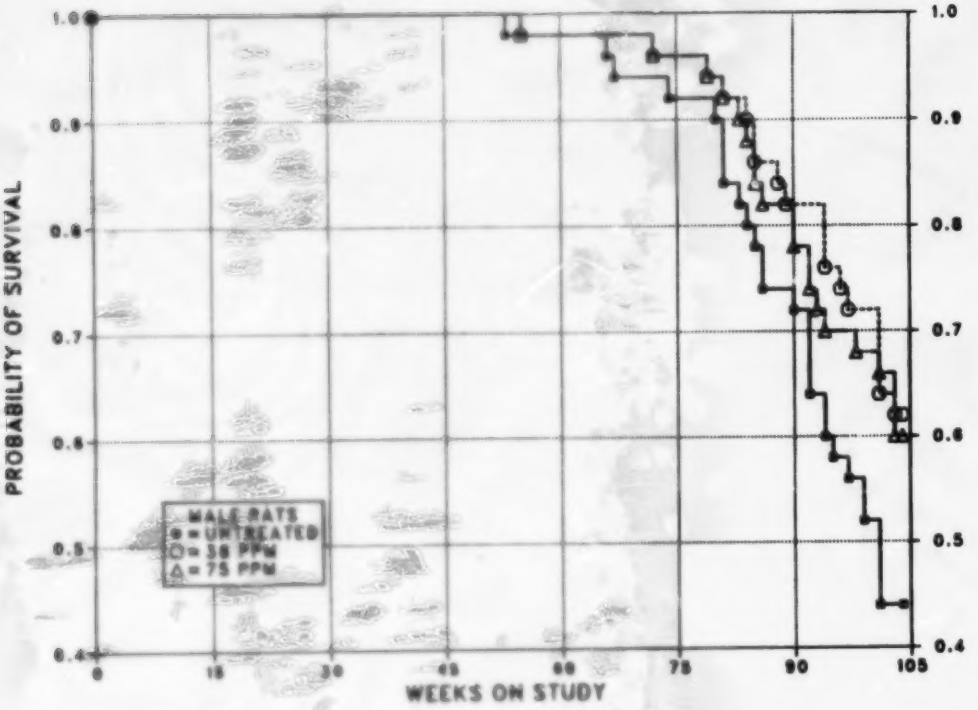
Lesions in male rats are summarized in Appendix A. Histopathologic findings on neoplasms in male rats are summarized in Table A1. Table A2 gives the survival and tumor status for individual male rats. Table A3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes). Historical incidences of tumors in control male rats are listed in Table A4. Findings on nonneoplastic lesions are summarized in Table A5.

Lesions in female rats are summarized in Appendix B. Histopathologic findings on neoplasms in female rats are summarized in Table B1. Table B2 gives the survival and tumor status for individual female rats. Table B3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table B3 (footnotes). Historical incidences of tumors in control female rats are listed in Table B4. Findings on nonneoplastic lesions are summarized in Table B5.

TABLE 14. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF ROTENONE

	Control	38 ppm	75 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	28	19	20
Killed at termination	22	31	30
Survival P values (c)	0.109	0.076	0.142
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	23	18	19
Killed at termination	27	32	31
Survival P values (c)	0.438	0.367	0.506

(a) Terminal-kill period: week 104
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.



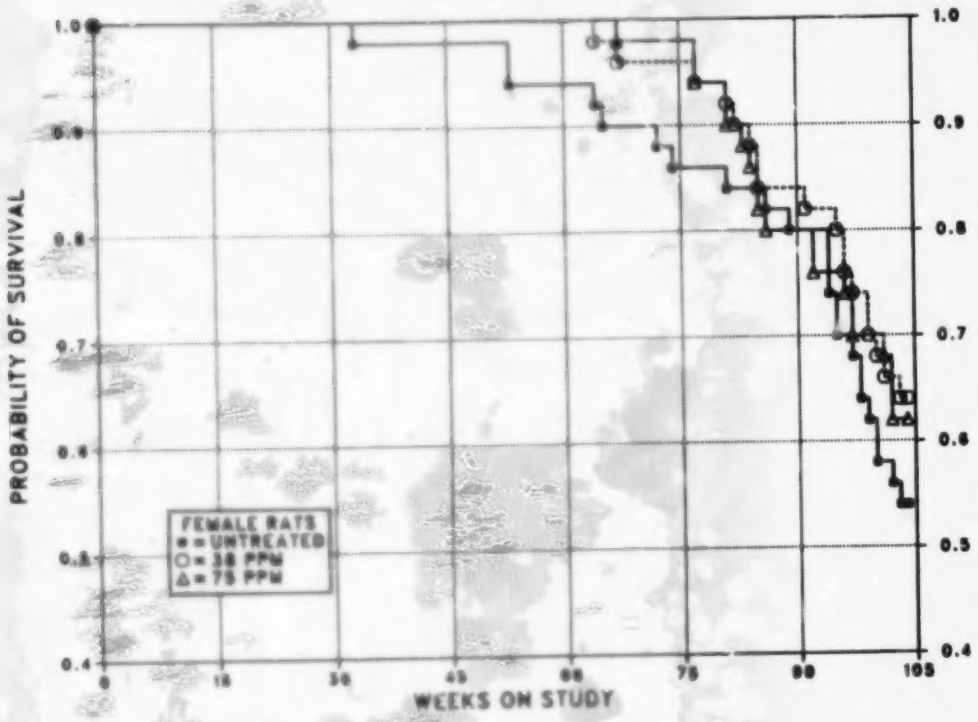


FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING ROTENONE FOR TWO YEARS

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Subcutaneous Tissue: The incidence of fibromas, neurofibromas, sarcomas, myxosarcomas, or fibrosarcomas (combined) in low dose female rats was significantly greater than that in the controls by the incidental tumor test (Table 15).

Parathyroid: Adenomas were observed in 1/41 control, 0/44 low dose, and 4/44 high dose male rats. The incidences of hyperplasia in this gland were 2/41, 1/44, and 4/44. The adenomas were not observed grossly, and they consisted of spherical masses of enlarged cells with vesicular nuclei which compressed slightly the adjacent normal tissue. The incidences in the dosed groups were not significantly different from that in the controls. The historical incidence in untreated control male rats in NTP studies is 4/1 314 (0.3%). No more than one adenoma has been observed in any control group.

Anterior Pituitary Gland: Focal hyperplasia was observed at an increased incidence in high dose male rats (control, 7/49, 14%; low dose, 2/15, 13%; high dose, 13/50, 26%), but the incidences of neoplasms in dosed male rats were not increased.

TABLE 15. ANALYSIS OF SUBCUTANEOUS TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (a)

	Control	38 ppm (b)	75 ppm (b)
Fibroma			
Overall Rates	0/50 (0%)	1/50 (2%)	0/50 (0%)
Neurofibroma			
Overall Rates	0/50 (0%)	1/50 (2%)	0/50 (0%)
Sarcoma			
Overall Rates	0/50 (0%)	1/50 (2%)	1/50 (2%)
Fibrosarcoma			
Overall Rates	0/50 (0%)	1/50 (2%)	2/50 (4%)
Myxosarcoma			
Overall Rates	0/50 (0%)	1/50 (2%)	0/50 (0%)
Fibroma, Neurofibroma, Sarcoma, Fibrosarcoma, or Myxosarcoma			
Overall Rates	0/50 (0%)	5/50 (10%)	3/50 (6%)
Adjusted Rates	0.0%	12.5%	7.1%
Terminal Rates	0/27 (0%)	2/32 (6%)	0/31 (0%)
Week of First Observation		64	77
Life Table Tests	P=0.163	P=0.049	P=0.143
Incidental Tumor Tests	P=0.067	P=0.013	P=0.091

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix B, Table B3 (footnotes).
(b) The estimated dose in milligrams per kilograms per day is given in Chapter III (Body Weights and Clinical Signs) and in Appendix G.

FIRST FOURTEEN-DAY STUDIES

All mice survived to the end of the studies (Table 16). Final mean body weights of male mice and feed consumption were not affected by incorporation of rotenone in the diet. No compound-related clinical signs or gross or microscopic pathologic effects were observed. Because rotenone was not toxic at the dietary concentrations tested, a second 14-day study with higher concentrations (up to 4,800 ppm) was conducted.

SECOND FOURTEEN-DAY STUDIES

No compound-related deaths occurred (Table 17). Final mean body weights of dosed and control mice were similar. Estimated feed consumption by dosed and control groups was similar. No compound-related clinical signs or gross pathologic effects were observed.

TABLE 16. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE FIRST FOURTEEN-DAY FEED STUDIES OF ROTENONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)	Feed Consumption (c) Week 1	Week 2
MALE							
0	5/5	28.4	31.4	+3.0	--	7.7	8.1
50	5/5	27.6	29.4	+1.8	93.6	7.9	7.9
100	5/5	28.4	30.6	+2.2	97.5	6.9	8.5
200	5/5	26.4	29.8	+3.4	94.9	8.5	6.5
400	5/5	27.4	29.4	+2.0	93.6	7.1	7.6
800	5/5	28.2	30.6	+2.4	97.5	7.6	7.6
FEMALE							
0	5/5	22.0	24.4	+2.4	--	8.3	8.9
50	5/5	21.2	23.8	+2.6	97.5	7.5	11.0
100	5/5	21.0	23.4	+2.4	95.9	8.2	8.4
200	5/5	22.0	23.8	+1.8	97.5	6.5	7.2
400	5/5	21.8	23.4	+1.6	95.9	7.3	8.7
800	5/5	21.4	22.8	+1.4	93.4	6.9	7.3

(a) Number surviving/number in group
(b) Mean body weight change of the group
(c) Grams of feed per animal per day

TABLE 17. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE SECOND FOURTEEN-DAY FEED STUDIES OF ROTENONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 1	Week 2
MALE							
0	5/5	25.8 ± 0.7	28.4 ± 1.2	+2.6 ± 0.5	--	7.0	7.6
300	5/5	24.8 ± 0.6	27.6 ± 0.5	+2.8 ± 0.4	97.2	7.3	7.6
600	5/5	23.4 ± 0.9	26.4 ± 0.5	+3.0 ± 0.8	93.0	7.3	8.1
1,200	5/5	25.4 ± 0.5	27.2 ± 0.7	+1.8 ± 0.4	95.8	7.8	8.4
2,400	5/5	25.8 ± 1.1	25.6 ± 1.7	−0.2 ± 0.9	90.1	7.1	7.2
4,800	5/5	25.8 ± 0.7	28.6 ± 0.5	+2.8 ± 0.7	100.7	7.6	7.8
FEMALE							
0	5/5	20.0 ± 0.3	22.8 ± 0.2	+2.8 ± 0.2	--	7.2	8.2
300	5/5	19.6 ± 0.6	22.4 ± 0.4	+2.8 ± 0.4	98.2	7.0	8.0
600	5/5	20.0 ± 0.5	22.6 ± 0.4	+2.6 ± 0.2	99.1	7.4	7.3
1,200	5/5	20.4 ± 0.5	21.8 ± 0.7	+1.4 ± 0.6	95.6	8.3	7.3
2,400	5/5	19.4 ± 0.5	21.2 ± 0.7	+1.8 ± 0.4	93.0	6.3	6.3
4,800	5/5	20.6 ± 0.5	22.2 ± 0.7	+1.6 ± 0.2	97.4	8.0	8.1

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Grams of feed per animal per day

THIRTEEN-WEEK STUDIES

All mice that received 50,000 ppm and 9/10 males and 8/10 females that received 16,000 ppm rotenone died before the end of the studies (Table 18). Final mean body weights of mice that received 5,000 or 16,000 ppm were 14% and 26% lower than that of the controls for males and 22% or 12% lower for females. Relative liver weights were significantly increased ($P<0.05$) in males and females at 600 ppm, 1,900 ppm, and 5,000 ppm (Table 19).

Dose Selection Rationale: Based on weight gain depression and observed mortality, doses selected for mice for the 2-year studies were 600 and 1,200 ppm rotenone in feed.

TABLE 18. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Feed Consumption (d) Week 4	Week 12
MALE							
0	(e) 9/10	23.6 ± 0.4	33.8 ± 0.6	+10.3 ± 0.3	--	7.3	6.9
600	10/10	22.6 ± 0.7	31.0 ± 0.8	+8.4 ± 0.6	91.7	7.3	8.0
1,900	10/10	24.3 ± 0.7	32.1 ± 0.5	+7.8 ± 0.7	95.0	7.6	8.1
5,000	(f) 9/10	24.0 ± 0.6	29.2 ± 0.5	+5.6 ± 0.4	86.4	8.3	10.9
16,000	(g) 1/10	24.5 ± 0.4	25.0 ± 0.0	+3.0 ± 0.0	74.0	--	--
50,000	(h) 0/10	24.4 ± 0.4	(i)	(i)	--	--	--
FEMALE							
0	10/10	19.2 ± 0.5	28.0 ± 1.0	+8.8 ± 0.7	--	6.8	10.4
600	10/10	19.0 ± 0.2	26.1 ± 0.4	+7.1 ± 0.3	93.2	7.3	7.7
1,900	10/10	18.2 ± 0.6	26.4 ± 0.7	+8.2 ± 0.5	94.3	8.2	7.5
5,000	10/10	17.5 ± 0.4	21.8 ± 1.1	+4.3 ± 1.1	77.9	7.2	9.3
16,000	(j) 2/10	19.5 ± 0.4	24.5 ± 2.5	+3.5 ± 1.5	87.5	--	--
50,000	(k) 0/10	19.3 ± 0.3	(i)	(i)	--	--	--

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Grams of feed per animal per day
(e) Week of death: 13
(f) Week of death: 3
(g) Week of death: 1,1,1,1,2,2,2,2,3
(h) Week of death: all 1
(i) No data are reported due to the 100% mortality in this group.
(j) Week of death: 1,2,2,2,2,2,2,2
(k) Week of death: 1,1,1,1,1,1,2,2,2,2

TABLE 19. LIVER WEIGHT TO BODY WEIGHT RATIOS OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF ROTENONE (a)

Concentration (ppm)	No. Examined	Necropsy Body Weight (grams)	Liver Weight (mg)	Liver Weight/Necropsy Body Weight (mg/g)
MALE				
0	9	30.8 ± 1.9	1,401 ± 106	45.6 ± 2.90
600	10	30.6 ± 2.2	(b) 1,915 ± 231	(b) 62.5 ± 5.91
1,900	10	32.2 ± 2.0	(b) 1,903 ± 393	(b) 59.2 ± 11.19
5,000	9	29.9 ± 1.6	(b) 1,877 ± 179	(b) 62.8 ± 5.36
FEMALE				
0	10	26.0 ± 3.3	1,183 ± 157	45.6 ± 3.24
600	10	25.9 ± 1.0	1,410 ± 135	(c) 54.4 ± 4.09
1,900	10	26.0 ± 1.9	(b) 1,516 ± 225	(b) 58.2 ± 6.67
5,000	(d) 10	25.8 ± 1.3	(b) 1,612 ± 305	(b) 62.4 ± 9.84
16,000	2	30.0 ± 2.8	1,555 ± 106	51.9 ± 1.36

(a) Mean ± SD; P values are versus the controls by Dunnett's test (Dunnett, 1955).
(b) $P<0.01$ relative to controls
(c) $P<0.05$ relative to controls
(d) Two final body weights not taken; data for mean body weight and ratio are for eight animals.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male mice were generally 5%-10% lower than those of the controls between week 4 and week 33 and 10%-19% lower from week 37 to the end of the study (Table 20 and Figure 5). Mean body weights of low dose male mice were 5%-13% lower than those of the controls between week 29 and the end of the study. Mean body weights of dosed female mice were 7%-30% lower than those of the controls from week 15 to the end of the study.

The estimated daily feed consumption by low dose and high dose male mice was 103% and 106% that of the controls and by low dose and high dose female mice, 113% and 115% that of the controls (Appendix G, Tables G3 and G4). The estimated amount of rotenone consumed per day was approximately 111 mg/kg or 242 mg/kg for low dose and high dose male mice and 124 mg/kg or 265 mg/kg for low dose and high dose female mice.

TABLE 20. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF ROTENONE

Weeks on Study	Control		600 ppm			1,200 ppm		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE								
0	23.3	50	23.6	98	50	22.9	98	50
1	25.3	50	25.6	101	50	24.9	98	50
2	27.5	50	26.6	97	50	27.0	98	50
3	26.4	50	26.7	101	50	26.7	101	50
4	26.6	50	27.5	103	50	25.3	95	50
5	26.8	50	26.5	99	50	24.2	91	50
6	29.6	50	29.1	98	50	28.0	94	50
7	30.4	50	29.8	98	50	27.4	90	50
8	29.6	50	28.9	98	50	28.0	95	50
11	30.6	49	29.4	96	50	28.8	94	50
13	31.6	49	31.7	100	50	30.7	97	50
15	32.0	48	31.6	99	49	29.7	93	50
20	33.3	44	31.8	96	49	30.2	93	50
24	34.9	44	34.1	98	49	32.1	92	50
29	37.0	42	34.1	92	48	32.8	89	50
33	37.8	42	35.6	94	48	34.0	90	50
37	39.1	39	35.8	92	48	34.4	88	50
42	38.2	39	36.3	95	48	34.4	90	49
47	40.0	39	37.3	93	47	35.4	89	49
51	40.7	38	38.0	93	47	35.7	88	49
56	41.1	38	38.1	93	45	35.2	86	49
60	41.0	38	37.7	92	45	35.2	86	49
64	41.9	38	37.5	89	44	35.7	85	49
69	42.4	38	37.5	88	44	35.2	83	49
73	42.4	37	36.5	86	44	34.6	82	49
77	44.0	36	38.1	87	44	35.8	81	49
81	42.7	36	37.3	87	43	35.1	82	49
87	41.3	35	37.0	90	43	35.6	86	49
91	42.6	33	36.7	86	40	34.7	81	49
94	41.1	31	36.4	89	40	35.2	86	49
99	37.9	30	35.7	94	37	33.9	89	47
103	38.9	29	35.6	92	37	34.0	87	47
FEMALE								
0	17.8	50	18.0	101	50	17.4	98	50
1	19.8	50	19.5	98	50	19.4	98	50
2	20.8	50	20.5	99	50	20.7	100	50
3	21.1	50	20.0	95	50	20.1	95	50
4	21.4	50	20.7	97	50	20.6	96	50
5	21.4	50	20.7	97	50	20.8	97	50
6	22.2	50	21.4	96	50	21.6	97	50
7	22.2	50	22.1	95	50	21.2	91	50
8	22.6	50	22.0	97	50	21.6	96	50
11	24.4	50	23.2	95	50	22.7	93	50
13	24.4	50	23.6	97	49	23.4	96	50
15	25.7	50	24.0	93	49	23.6	92	50
20	27.6	50	25.4	92	49	24.7	89	50
24	30.0	50	27.0	90	49	27.0	90	50
29	30.7	50	27.8	91	49	26.6	87	50
33	31.4	50	27.6	88	49	26.3	84	50
37	32.9	50	28.9	88	49	27.4	83	50
42	33.2	50	29.4	89	49	27.9	84	50
47	35.6	49	30.9	84	49	28.5	80	50
51	36.5	48	31.8	87	49	29.7	81	50
56	37.5	48	31.6	84	48	29.4	78	50
60	37.8	48	33.0	86	48	29.7	80	50
64	38.7	48	32.8	85	48	30.7	79	49
69	38.9	47	32.1	80	47	29.7	74	49
73	41.1	46	33.1	78	46	28.6	70	49
77	41.6	45	34.4	83	44	31.0	75	49
81	41.1	44	33.6	82	44	31.0	75	49
87	43.5	43	35.4	81	43	32.4	74	48
91	44.5	42	35.4	80	42	32.0	72	48
94	44.2	42	35.6	81	42	32.7	74	48
99	44.6	39	36.1	81	42	33.1	74	45
103	42.6	37	35.5	83	42	32.3	76	45

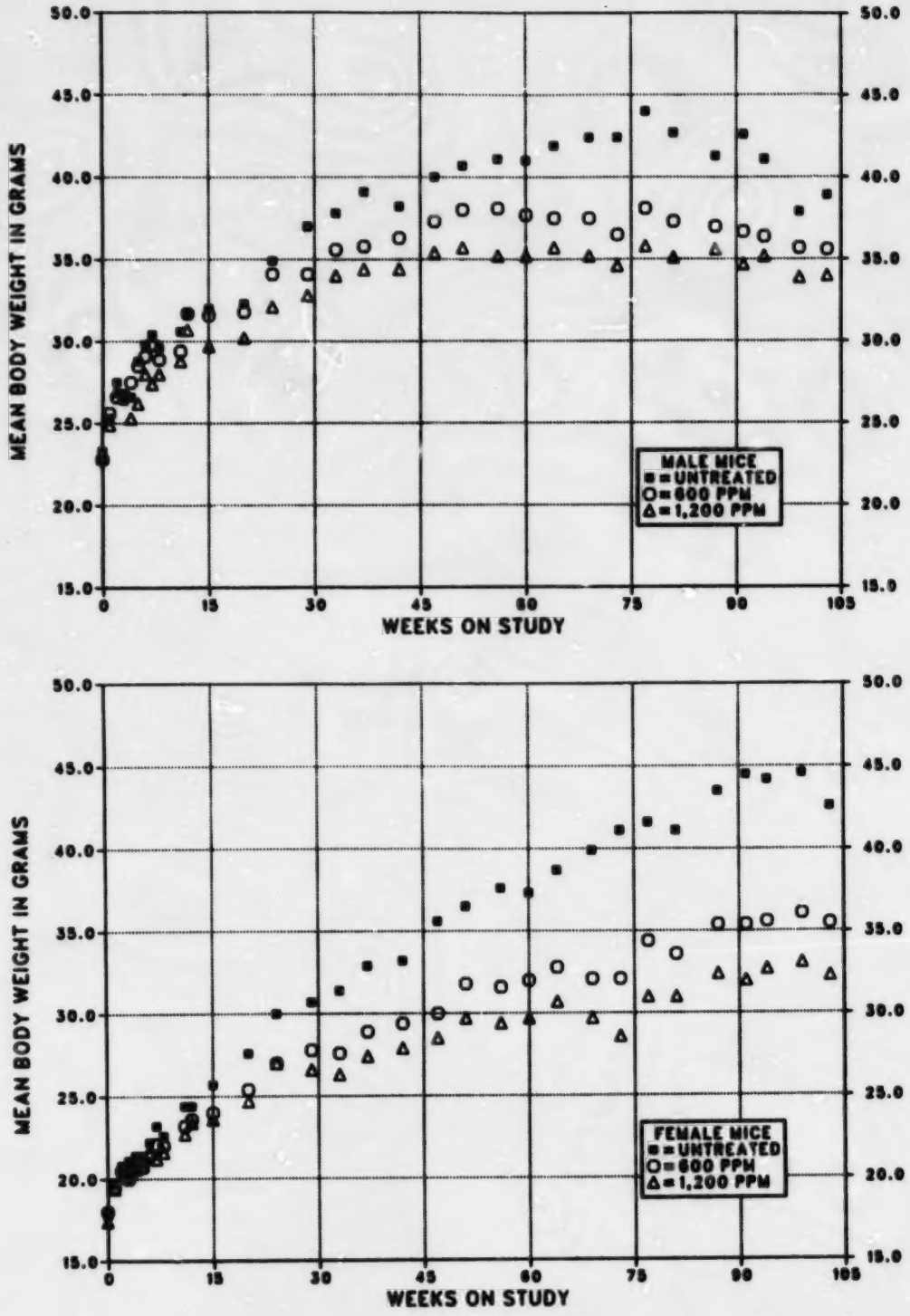

FIGURE 5. GROWTH CURVES FOR MICE FED DIETS CONTAINING ROTENONE FOR TWO YEARS

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Survival

Estimates of the probabilities of survival for male and female mice fed diets containing rotenone at the concentrations used in these studies and for controls are shown in Table 21 and in the Kaplan and Meier curves in Figure 6. The survival of the low dose group of male mice was significantly lower than that of the high dose group (P=0.007). The survival of the high dose group of male mice was significantly greater than that of the controls after week 27. No significant differences in survival were observed between any groups of female mice.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the liver and subcutaneous tissue.

Lesions in male mice are summarized in Appendix C. Histopathologic findings on neoplasms in male mice are summarized in Table C1. Table C2 gives the survival and tumor status for individual male mice. Table C3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table C3 (footnotes). Historical incidences of tumors in control male mice are listed in Table C4. Findings on nonneoplastic lesions are summarized in Table C5.

Lesions in female mice are summarized in Appendix D. Histopathologic findings on neoplasms in female mice are summarized in Table D1. Table D2 gives the survival and tumor status for individual female mice. Table D3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table D3 (footnotes). Findings on nonneoplastic lesions are summarized in Table D4.

TABLE 21. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF ROTENONE

	Control	600 ppm	1,200 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	21	14	3
Killed at termination	29	36	47
Survival P values (c)	<0.001	0.143	<0.001
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	12	7	5
Animals missing	1	1	0
Killed at termination	37	40	45
Died during termination period	0	2	0
Survival P values (c)	0.071	0.351	0.093

(a) Terminal-kill period: week 104
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the control column, and the results of the life table pairwise comparisons with the controls are in the dosed columns.

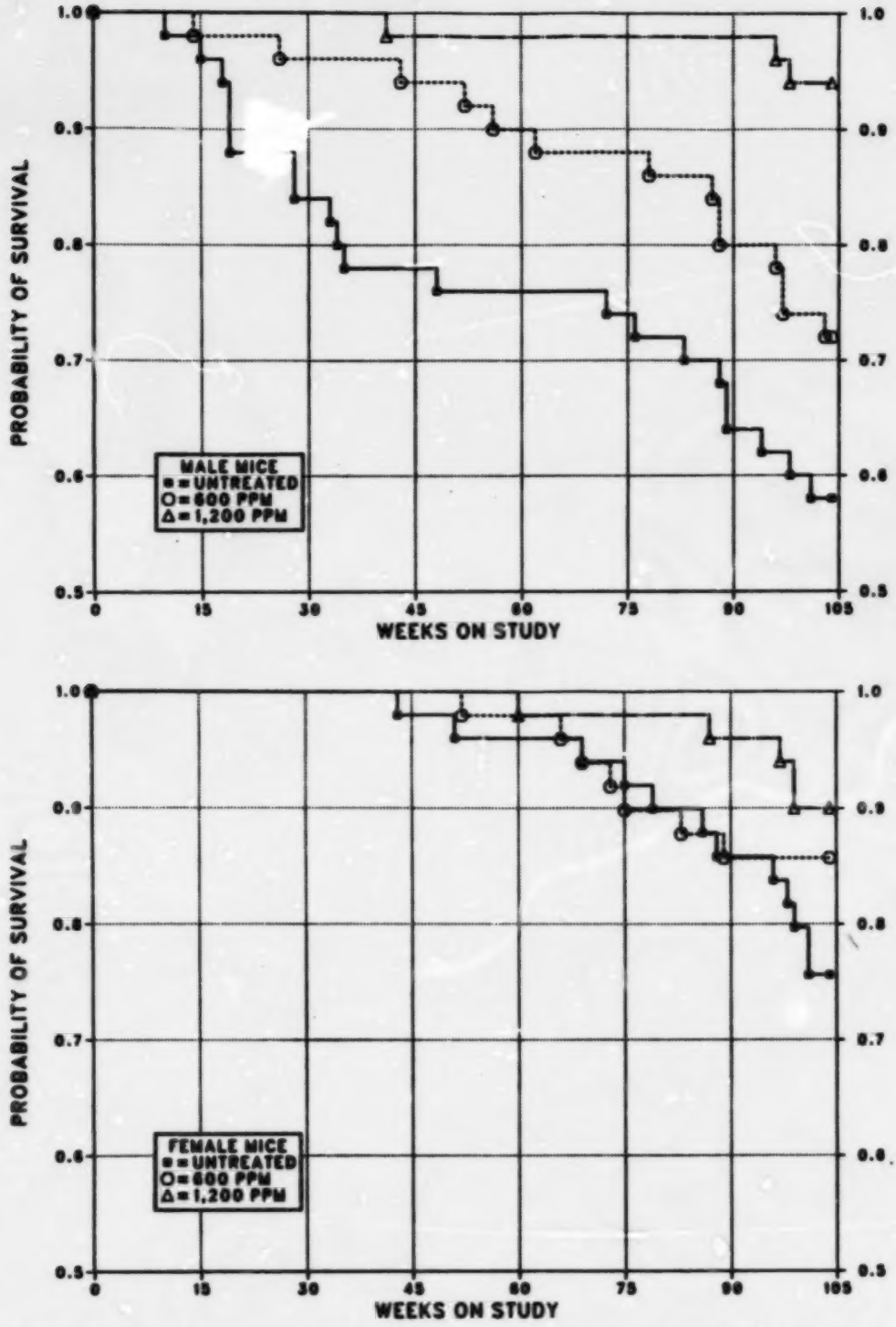

FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING ROTENONE FOR TWO YEARS

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Liver: Hepatocellular adenomas, carcinomas, and adenomas or carcinomas (combined) occurred with significant negative trends in male mice, and the incidences in the high dose group were significantly lower than those in the controls (Table 22).

Subcutaneous Tissue: Fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) in male mice occurred with a significant negative trend (control, 8/49, 16%; low dose, 4/50, 8%; high dose, 2/50, 4%; $P<0.05$), and the incidence in the high dose group was significantly lower than that in the controls by the life table test ($P=0.01$).

TABLE 22. ANALYSIS OF LIVER TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (a)

	Control	600 ppm (b)	1,200 ppm (b)
Hepatocellular Adenoma			
Overall Rates	7/47 (15%)	9/49 (18%)	1/50 (2%)
Adjusted Rates	22.2%	23.3%	2.1%
Terminal Rates	5/29 (17%)	7/36 (19%)	1/47 (2%)
Week of First Observation	89	88	104
Life Table Tests	P=0.005N	P=0.575	P=0.006N
Incidental Tumor Tests	P=0.019N	P=0.532	P=0.018N
Hepatocellular Carcinoma			
Overall Rates	6/47 (13%)	3/49 (6%)	0/50 (0%)
Adjusted Rates	18.5%	7.8%	0.0%
Terminal Rates	4/29 (14%)	2/36 (6%)	0/47 (0%)
Week of First Observation	76	87	
Life Table Tests	P=0.002N	P=0.157N	P=0.004N
Incidental Tumor Tests	P=0.009N	P=0.184N	P=0.019N
Hepatocellular Adenoma or Carcinoma (c)			
Overall Rates	12/47 (26%)	12/49 (24%)	1/50 (2%)
Adjusted Rates	35.6%	30.3%	2.1%
Terminal Rates	8/29 (28%)	9/36 (25%)	1/47 (2%)
Week of First Observation	76	87	104
Life Table Tests	P<0.001N	P=0.365N	P<0.001N
Incidental Tumor Tests	P=0.001N	P=0.429N	P=0.001N

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix C, Table C3 (footnotes).
(b) The estimated dose in milligrams per kilograms per day is given in Chapter III (Body Weights and Clinical Signs) and in Appendix G.
(c) Historical incidence at study laboratory (mean ± SD): 121/397 (31% ± 7%); historical incidence in NTP studies: 627/2,084 (30% ± 8%)

IV. DISCUSSION AND CONCLUSIONS

Short-Term Studies
Two-Year Studies: Rats
Two-Year Studies: Mice
Genotoxicity Studies
Data Audit

IV. DISCUSSION AND CONCLUSIONS

Toxicology and carcinogenesis studies were conducted by giving feed containing rotenone to F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years.

Short-Term Studies

In the first 14-day feed studies, no toxicity was observed in rats and mice fed diets containing 50, 100, 200, 400, or 600 ppm rotenone. In the second 14-day feed studies conducted at dietary concentrations up to 4,800 ppm, rotenone was toxic to rats but not to mice. Toxic effects observed in rats included deaths at 2,400 ppm and body weight depression relative to controls of greater than 10% at concentrations of 600 ppm or higher. Male and female rats fed 1,200 ppm or more lost weight. The weight loss ranged from 16% of the initial body weight for male rats administered 1,200 ppm to 43% for rats receiving 2,400 ppm. Female rats receiving 1,200 ppm rotenone or higher lost 20% of their initial body weight. This weight loss was attributed to reduced feed consumption, possibly caused by poor palatability of formulated diets. Feed consumption by male rats given rotenone at 1,200 ppm or 4,800 ppm was 65% or 45% that of controls; at the same concentrations, feed consumption by female rats was 90% and 65% that of control values. Feed consumption by mice receiving rotenone was similar to that of controls.

In the 13-week studies, reductions in mean body weight greater than 15% were noted in male rats receiving concentrations of 300 ppm or more and in female rats given more than 150 ppm; females in the 600- or 1,200-ppm groups lost weight. In mice, reductions in mean body weight of more than 10% were observed in males and females receiving 5,000 ppm or higher. Deaths occurred in rats administered rotenone at concentrations of 600 ppm or higher and in mice at concentrations of 5,000 ppm (males) or 16,000 ppm or higher (females).

Sites in rats affected by rotenone administration for 13 weeks included bone marrow, forestomach, and possibly the liver. Bone marrow atrophy, with generally dose-related increases in incidence and severity, was observed in male rats given 300 ppm or higher and in females receiving 75 ppm or higher; none was seen in controls (see Table 11). Similarly, inflammation and hyperplasia of the forestomach occurred with dose-related increased incidences and severity in males receiving 300 ppm or higher and in females administered 150 ppm or higher (see Table 12). Although relative liver weights were increased in male rats receiving 300 ppm and in females receiving 600 or 1,200 ppm, no structural changes were observed by light microscopy. The increased relative liver weight in dosed rats may be related to their reduced body weight. The absolute liver weights of these dose groups did not differ from those of the controls. Liver enlargement was previously observed in female Osborne-Mendel rats fed diets containing 100 ppm cube powder (rotenone content 5.8%) for 2 years (Hansen et al., 1965).

No compound-related lesions were identified in mice. Increased relative liver weights were observed in mice fed diets containing 600, 1,900, or 5,000 ppm rotenone. These increases appear to be associated with increased absolute liver weights (see Table 19).

Two-Year Studies: Rats

Administration of rotenone at concentrations of 38 or 75 ppm in the diet for 2 years did not adversely affect the survival of rats (male: control, 22/50; low dose, 31/50; high dose, 30/50; female: control, 27/50; low dose, 32/50; high dose, 31/50). Body weights and feed consumption of dosed rats were similar to those of the controls. Survival and body weight data suggest that doses administered during the 2-year studies in rats were reasonable.

Adenomas of the parathyroid gland occurred in 1/41 control, 0/44 low dose, and 4/44 high dose male rats. Although not statistically significant, the incidence in the high dose group greatly exceeds the historical incidence in untreated control male rats (4/1,314, 0.3%). The biologic behavior of this proliferative lesion is unknown. Carcinomas of the parathyroid have not occurred in NTP historical untreated control male F344/N rats, nor does morphologic evidence exist for progression from adenoma to carcinoma. Parathyroid adenoma is distinguished from hyperplasia by its focal nature and compression of adjacent normal tissue. Parathyroid

hyperplasia is relatively much more common in male rats and generally occurs secondary to severe renal disease (spontaneous progressive nephropathy); whether parathyroid adenoma is related to this process is unknown. The unusually high incidence of parathyroid adenoma in high dose male rats may be related to the administration of rotenone. However, the severity of renal disease in the four high dose male rats with parathyroid adenomas was not marked.

In the present study, focal hyperplasia of the anterior pituitary gland occurred with an increased incidence in high dose male rats (control, 7/49, 14%; low dose, 2/15, 13%; high dose, 13/50, 26%), but the incidence of tumors of the anterior pituitary gland did not increase.

The incidences of subcutaneous tissue fibromas, fibrosarcomas, sarcomas, myxosarcomas, or neurofibrosarcomas (combined) in dosed male rats were not significantly different from that in the controls (control, 5/50, 10%; low dose, 3/50, 6%; high dose, 2/50, 4%). The incidence of these tumors in the low dose females was greater ($P<0.05$) than that in the controls (0/50; 5/50, 10%; 3/50, 6%). These tumors were combined because of their possible common histiogenic origin from fibroblasts or undifferentiated mesenchymal cells. The incidence of these tumors in the low dose females was greater than the historical rate at this laboratory (9/337, 3% ± 1%) and throughout the Program (50/2,021, 2% ± 2%).

A slight increase in the combined incidence of subcutaneous tissue tumors (fibromas or fibrosarcomas) was observed in male Wistar rats given 1.7 or 3.0 mg/kg rotenone either by gavage in corn oil or by intraperitoneal injection in studies conducted for the U.S. Environmental Protection Agency (Freudenthal et al., 1981). The incidences were as follows: gavage study--control, 0/25; low dose, 1/25 (4%); high dose, 3/25 (12%); intraperitoneal injection study--0/15; 2/25 (8%); 0/25. Because of the lack of a significant dose-related trend in the NTP studies and because statistical significance was attained only by combining tumors of differing morphology, the increased incidence of subcutaneous tissue tumors in low dose female rats was not considered to be related to administration of rotenone.

Two-Year Studies: Mice

In the present studies, final mean body weights were depressed in the groups of mice fed diets containing 600 or 1,200 ppm rotenone for 2 years. Final mean body weights were 92% and 87% that of the control value for low and high dose males and 83% and 76% for low and high dose females. The survival of high dose male mice was greater ($P<0.001$) than that of the controls (see Table 21).

Hepatocellular adenomas, carcinomas, and adenomas or carcinomas (combined) occurred in male mice with significant negative trends ($P<0.02$), and the incidences in the high dose groups were significantly lower ($P<0.02$) than those in the controls (hepatocellular adenomas or carcinomas [combined]: control, 12/47; low dose, 12/49; high dose, 1/50) (see Table 22).

Fibromas, sarcomas, fibrosarcomas, or neurofibrosarcomas (combined) of the subcutaneous tissue in male mice occurred with a negative trend ($P<0.05$), and the incidence in the high dose group was lower ($P=0.01$ by the life table test) than that in the controls (control, 8/49; low dose, 4/50; high dose, 2/50). This decreased incidence of subcutaneous tumors is not considered to be directly related to rotenone administration but may be associated with body weight depressions in the dosed animals. Association between reduced body weight and decreased tumor incidences has been reported by other investigators (Haseman, 1983; Tarone et al., 1981).

Genotoxicity Studies

Rotenone's genotoxic activity includes the induction of forward mutations in mouse L5178Y lymphoma cells in the absence of metabolic activation and an equivocal response in the assay for SCEs in CHO cells in the presence of rat liver S9 (Appendix E, Tables E2 and E3). An indication of possible indirect genotoxicity through interference with microtubular assembly and depletion of cellular ATP pools was reported by Barham and Brinkley (1976a,b). Assays for gene mutation in bacteria (Ashwood-Smith et al., 1972; Ficsor and LoPiccolo, 1972; Probst and Hill, 1980; Probst et al., 1981; Shirasu et al.,

1981; Moriya et al., 1983; Table E1), unscheduled DNA synthesis in mammalian cell cultures (Ahmed et al., 1977; Probst and Hill, 1980; Probst et al., 1981), and induction of chromosomal aberrations in CHO cells (Table E4) were uniformly negative.

Data Audit

The experimental and tabulated data for the NTP Technical Report on rotenone were examined for accuracy, consistency, and compliance with Good Laboratory Practice requirements. As summarized in Appendix I, the audit revealed no major problems with the conduct of the studies or with the collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity*[a] of rotenone for male F344/N rats, as indicated by an increased incidence of parathyroid gland adenomas (uncommon tumors). There was *no evidence of carcinogenic activity* in female F344/N rats fed diets containing 38 or 75 ppm rotenone. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice fed diets containing 600 or 1,200 ppm rotenone for 2 years. The decreased incidence of liver neoplasms in male mice may have been related to the administration of rotenone.

[a]Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 10-11.

V. REFERENCES

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1. Ahmed, F.; Hart, R.; Lewis, N. (1977) Pesticide induced DNA damage and its repair in cultured human cells. Mutat. Res. 42:161-174.

2. Allaben, W.; Burger, G.; Kodell, R. (1984) Bioassay for carcinogenicity of rotenone in female Wistar rats (draft).

3. Ambrose, A.; Haag, H. (1937) Toxicological studies of derris. Comparative toxicity and elimination of some constituents of derris. Ind. Eng. Chem. 29:429-431.

4. Ambrose, A.; DeEds, F.; McNaught, J. (1942) Chronic toxicity of derris and rotenone. Ind. Eng. Chem. 34:684-689.

5. Armitage, P. (1971) Statistical Methods in Medical Research. New York: John Wiley & Sons Inc., pp. 362-365.

6. Ashwood-Smith, M.; Trevino, J.; Ring, R. (1972) Mutagenicity of dichlorvos. Nature 240:418-420.

7. Barham, S.; Brinkley, B. (1976a) Action of rotenone and related respiratory inhibitors on mammalian cell division. 1. Cell kinetics and biochemical aspects. Cytobios 15:85-96.

8. Barham, S.; Brinkley, B. (1976b) Action of rotenone and related respiratory inhibitors on mammalian cell division. 2. Ultrastructural studies. Cytobios 15:97-109.

9. Berenblum, I., Ed. (1969) Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2. Geneva: International Union Against Cancer.

10. Boguslawski, W.; Zelewski; L. (1971) Inhibition of cholesterol biosynthesis by the respiratory chain inhibitors in human placenta and in rat liver. Biochem. Pharmacol. 20:3431.

11. Boorman, G.; Montgomery, C., Jr.; Eustis, S.; Wolfe, M.; McConnell, E.; Hardisty, J. (1985) Quality assurance in pathology for rodent carcinogenicity studies. Milman, H.; Weisburger, E., Eds.: Handbook of Carcinogen Testing. Park Ridge, NJ: Noyes Publications, pp. 345-357.

12. Chemical Economics Handbook (1984) Insecticides, Sections 573.3005F,G and 573.3006T,U. SRI International, Menlo Park, CA.

13. Clive, D.; Johnson, K.; Spector, J.; Batson, A.; Brown, M. (1979) Validation and characterization of the L5178Y/TK$^{+/-}$ mouse lymphoma mutagen assay system. Mutat. Res. 59:61-108.

14. Cox, D. (1972) Regression models and life tables. J. R. Stat. Soc. B34:187-220.

15. De Brabander, M.; Van de Veire, R.; Aerts, F.; Geuens, S.; Hoebeke, J. (1976) New culture model facilitating rapid quantitative testing of mitotic spindle inhibition in mammalian cells. J. Natl. Cancer Inst. 56:357-363.

16. Dunnett, C. (1955) A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50:1096-1122.

17. Ernster, L.; Dallner, C.; Azzone G. (1963) Differential effects of rotenone and amytal on mitochondrial electron and energy transfer. J. Biol. Chem. 238:1124-1131.

18. Farm Chemicals Handbook (1982) Willoughby, OH: Meister Publishing Co., p. C253.

19. Feinberg, R.; Turrki, P.; Witkowski, P. (1967) The effect of nicotinamide adenine dinucleotide and rotenone on oxidation of choline by rat liver mitochondria. J. Biol. Chem. 242:4614-4618.

20. Ficsor, G.; LoPiccolo, G. (1972) Survey of pesticides for mutagenicity by the bacterial-plate assay method. EMS Newslett. 6:6-8.

21. Freudenthal, R.; Leber, A.; Thake, D.; Baron, R. (1981) Project Summary--Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters. EPA-600/S1-81-037. Research Triangle Park, NC: Environmental Protection Agency Health Effects Research Laboratory.

22. Fukami, J.-I.; Shishido, T.; Fukunaga, K.; Casida, J. (1969) Oxidative metabolism of rotenone in mammals, fish and insects and its relation to selective toxicity. J. Agric. Food Chem. 17:1217-1226.

23. Galloway, S.; Bloom, A.; Resnick, M.; Margolin, B.; Nakamura, F.; Archer, P.; Zeiger, E. (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. 7:1-51.

24. Gart, J.; Chu, K.; Tarone, R. (1979) Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62:957-974.

25. Gosalvez, M. (1983) Minireview: Carcinogenesis with the insecticide rotenone. Life Sci. 32:809-816.

26. Gosalvez, M.; Merchan, J. (1973) Induction of rat mammary adenomas with the respiratory inhibitor rotenone. Cancer Res. 33:3047-3050.

27. Haag, H. (1933) A contribution to the pharmacology of anabasine. J. Pharmacol. Exp. Ther. 48:95-104.

28. Hansen, W.; Davis, K.; Fitzhugh, O. (1965) Chronic toxicity of cube. Toxicol. Appl. Pharmacol. 7:535-542.

29. Haseman, J. (1983) Patterns of tumor incidence in two-year cancer bioassay feeding studies in Fischer 344 rats. Fundam. Appl. Toxicol. 3:1-9.

30. Haseman, J. (1984) Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58:385-392.

31. Haseman, J.; Huff, J.; Boorman, G. (1984) Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126-135.

32. Haseman, J.K.; Huff, J.; Rao, G.N.; Arnold, J.; Boorman, G.A.; McConnell, E.E. (1985) Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. J. Natl. Cancer Inst. 75:975-984.

33. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. Suppl. 1:3-142.

34. Hayes, W. (1982) Rotenone and related materials. Pesticides Studied in Man. Baltimore: Williams & Wilkins Co., pp. 81-86.

35. Hilton, J.; Walker, M. (1977) DNA strand scission and its repair following exposure of cells to inhibitors of oxidative phosphorylation. Biochem. Biophys. Res. Commun. 75:909-914.

36. Hull, F.; Whereat, A. (1967) The effect of rotenone on the regulation of fatty acid synthesis in heart mitochondria. J. Biol. Chem. 242:4023-4028.

37. Innes, R.; Ulland, B.; Valerio, M.; Petrocelli, L.; Fishbein, L.; Hart, E.; Pallotta, A.; Bates, R. (1969) Bioassays of pesticides and industrial chemicals for tumorigenicity in mice. J. Natl. Cancer Inst. 42:1101-1114.

38. Kaplan, E.; Meier, P. (1958) Nonparametric estimation of incomplete observations. J. Am. Stat. Assoc. 53:457-481.

39. Khera, K.; Whalen, C.; Angers, G. (1982) Teratogenicity study on pyrethrum and rotenone (natural origin) and ronnel in pregnant rats. J. Toxicol. Environ. Health 10:111-119.

40. Kline, D.; Hanna, G.; Honaker, C., Kuhn, G.; Jameson, C. (1986) Preparation and stability of animal feed mixtures dosed with rotenone. J. Assoc. Off. Anal. Chem. 69:600-663.

41. Leber, A.; Persing, R. (1979) Carcinogenic Potential of Rotenone, Phase I: Dietary Administration to Hamsters. EPA-600/1-79-004a. Research Triangle Park, NC: Environmental Protection Agency Health Effects Research Laboratory.

42. Lindahl, P.; Oberg, K. (1961) The effect of rotenone on respiration and its point of attack. Exp. Cell Res. 23:228.

V. REFERENCES

43. Linhart, M.; Cooper, J.; Martin, R.; Page, N.; Peters, J. (1974) Carcinogenesis Bioassay Data System. Comput. Biomed. Res. 7:230-248.

44. Mantel, N.; Haenszel, W. (1959) Statistical aspects of the analysis of data from retrospective studies of disease. J. Natl. Cancer Inst. 22:719-748.

45. Maronpot, R.; Boorman, G. (1982) Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol. Pathol. 10:71-80.

46. McConnell, E. (1983a) Pathology requirements for rodent two-year studies. I. A review of current procedures. Toxicol. Pathol. 11:60-64.

47. McConnell, E. (1983b) Pathology requirements for rodent two-year studies. II. Alternative approaches. Toxicol. Pathol. 11:65-76.

48. McConnell, E.; Solleveld, H.; Swenberg, J.; Boorman, G. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76:283-289.

49. Meisner, H.; Sorensen, L. (1966) Metaphase arrest of Chinese hamster cells with rotenone. Exp. Cell Res. 41:291-295.

50. Merck Index (1983) Rotenone. Rahway, NJ: Merck & Co., Inc., p. 1191.

51. Miyano, M. (1965) Rotenoids. XX. Total synthesis of rotenone. J. Am. Chem. Soc. 87:3958-3962.

52. Moriya, M.; Ohta, K.; Watanabe, T.; Miyazawa, K.; Kato, K.; Shirasu, Y. (1983) Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. 116:185-216.

53. Myhr, B.; Bowers, L.; Caspary, W. (1985) Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. Prog. Mutat. Res. 5:555-568.

54. National Cancer Institute (NCI) (1976) Guidelines for Carcinogen Bioassay in Small Rodents. NCI Carcinogenesis Technical Report Series No. 1. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health.

55. National Institute for Occupational Safety and Health (NIOSH) (1983) Registry of Toxic Effects of Chemical Substances (RTECS), Vol. 1. Tatkin, R.; Lewis, R., Sr., Eds. Cincinnati, OH: NIOSH, p. 645.

56. National Institutes of Health (NIH) (1978) Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

57. Oberg, K. (1961) Site of action of rotenone in the respiration chain. Exp. Cell Res. 24:163-164.

58. Oliver, W.; Roe, C. (1957) Rotenone poisoning of swine. J. Am. Vet. Assoc. 130:410-411.

59. Probst, G.; Hill, L. (1980) Chemically-induced DNA repair synthesis in primary rat hepatocytes: A correlation with bacterial mutagenicity. Ann. N. Y. Acad. Sci. 349:405-406.

60. Probst, G.; McMahon, R.; Hill, L.; Thompson, C.; Epp, J.; Neal, S. (1981) Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ. Mutagen. 3:11-32.

61. Santi, R.; Toth, C. (1965) Toxicology of rotenone. Farmaco. Ed. Sci. 20:270.

62. Santi, R.; Ferrari, M.; Toth, E. (1963) Papaverine-like pharmacological properties of rotenone. J. Pharm. Pharmacol. 15:697.

63. Santi, R.; Ferrari, M.; Toth, E. (1966) Pharmacological properties of rotenone. Farmaco. Ed. Sci. 20:689-703.

64. Shirasu, Y.; Moriya, M.; Tezuka, H.; Teramoto, S.; Ohta, T.; Inoue, T. (1981) Mutagenicity screening studies on pesticides. Environmental Mutagens and Carcinogens, Proceedings, 3rd International Conference on Environmental Mutagens, Tokyo, Mishima, and Kyoto, Sept. 21-27, pp. 331-335.

65. Spencer, F.; Sing, L. (1982) Reproductive responses to rotenone during decidualized pseudogestation and gestation in rats. Bull. Environ. Contam. 28:360-368.

66. Tarone, R. (1975) Tests for trend in life table analysis. Biometrika 62:679-682.

67. Tarone, R.; Chu, K.; Ward, J. (1981) Variability in rates of some naturally occurring tumors in F344 rats and B6C3F$_1$ mice. J. Natl. Cancer Inst. 66:1175-1181.

68. Tomkins, D.; Kwok, E.; Douglas, G. (1980) Testing of pesticides for induction of sister chromatid exchange in Chinese hamster ovary cells. Can. J. Cenet. Cytol. 22:681.

69. Unai, T.; Cheng, H.; Yamamoto, I.; Casida, J. (1973) Chemical and biological *O,O*-demethylation of rotenone derivatives. Agric. Biol. Chem. 37:1937-1944.

70. U.S. Environmental Protection Agency (USEPA) (1980) Index to Pesticide Chemicals IV-071003; 3-21-80.

71. Yamamoto, I.; Casida, J. (1967) Metabolism of Rotenone by Mammals and Insects. Presented at Am. Chem. Soc. week of April 10.

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS

IN THE TWO-YEAR FEED STUDY OF ROTENONE

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TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
Basal cell tumor		1 (2%)	
Trichoepithelioma		1 (2%)	
Sebaceous adenoma		1 (2%)	
Keratoacanthoma	1 (2%)	2 (4%)	
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma	4 (8%)	1 (2%)	
Fibrosarcoma	1 (2%)	2 (4%)	
Myxosarcoma			1 (2%)
Lipoma	1 (2%)		1 (2%)
Neurofibrosarcoma			1 (2%)
RESPIRATORY SYSTEM			
#Lung	(50)	(12)	(50)
Alveolar/bronchiolar adenoma			1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	24 (48%)	20 (40%)	25 (50%)
#Spleen	(49)	(26)	(50)
Sarcoma, NOS	1 (2%)		
Mesothelioma, NOS		1 (4%)	
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		
#Salivary gland	(50)	(11)	(50)
Squamous cell carcinoma, unclear prim or meta		1 (9%)	
#Liver	(50)	(23)	(50)
Neoplastic nodule	3 (6%)		2 (4%)
Hepatocellular carcinoma		1 (4%)	1 (2%)
#Pancreas	(49)	(11)	(50)
Acinar cell adenoma			1 (2%)
#Forestomach	(49)	(8)	(49)
Squamous cell papilloma		1 (13%)	
URINARY SYSTEM			
#Kidney	(50)	(12)	(50)
Tubular cell adenocarcinoma	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#Anterior pituitary	(49)	(15)	(50)
Carcinoma, NOS		1 (7%)	
Adenoma, NOS	17 (35%)	6 (40%)	16 (32%)
#Adrenal medulla	(50)	(13)	(50)
Pheochromocytoma	21 (42%)	6 (46%)	24 (48%)
Pheochromocytoma, malignant			1 (2%)
#Thyroid	(50)	(49)	(49)
Follicular cell adenoma		1 (2%)	
Follicular cell carcinoma	1 (2%)		4 (8%)
C-cell adenoma	11 (22%)	3 (6%)	7 (14%)
C-cell carcinoma		2 (4%)	2 (4%)
#Parathyroid	(41)	(44)	(44)
Adenoma, NOS	1 (2%)		4 (9%)
#Pancreatic islets	(49)	(11)	(50)
Islet cell adenoma	1 (2%)		3 (6%)
Islet cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS		1 (2%)	1 (2%)
Fibroadenoma	2 (4%)	1 (2%)	
*Preputial gland	(50)	(50)	(50)
Carcinoma, NOS	2 (4%)	3 (6%)	1 (2%)
Adenoma, NOS	7 (14%)	4 (8%)	4 (8%)
#Testis	(50)	(49)	(50)
Interstitial cell tumor	43 (86%)	47 (96%)	48 (96%)
Mesothelioma, NOS		1 (2%)	1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Nasolacrimal duct	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)		
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
*Maxilla	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)
*Scapula	(50)	(50)	(50)
Osteoma			1 (2%)
BODY CAVITIES			
*Abdominal cavity	(50)	(50)	(50)
Myxosarcoma, invasive			1 (2%)
*Tunica vaginalis	(50)	(50)	(50)
Mesothelioma, NOS		1 (2%)	
Mesothelioma, malignant	1 (2%)		
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Fibrosarcoma, metastatic		1 (2%)	
Mesothelioma, metastatic	1 (2%)		

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	9	4	4
Moribund sacrifice	19	15	16
Terminal sacrifice	22	31	30
TUMOR SUMMARY			
Total animals with primary tumors**	50	50	49
Total primary tumors	149	109	154
Total animals with benign tumors	49	49	49
Total benign tumors	110	75	110
Total animals with malignant tumors	30	26	31
Total malignant tumors	36	30	41
Total animals with secondary tumors##	1	1	1
Total secondary tumors	1	1	1
Total animals with tumors uncertain--benign or malignant	3	2	3
Total uncertain tumors	3	3	3
Total animals with tumors uncertain--primary or metastatic		1	
Total uncertain tumors		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE: UNTREATED CONTROL

ANIMAL NUMBER	0021	0017	0012	0018	0024	0009	0035	0043	0014	0038	0030	0007	0032	0011	0010	0013	0028	0048	0019	0015	0005	0004	0037	0042	0003
WEEKS ON STUDY	0558	0668	0677	0674	0682	0691	0691	0691	0693	0694	0695	0696	0696	0696	0696	0696	0696	0696	0696	0696	0696	0697	0698	0698	1001
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma									X		X														
Fibrosarcoma																									
Lipoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																							X		
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																			X						
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	–	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	+	–	–	–	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Stomach	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma																									
Urinary bladder	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS	X	X			X		X		X	X			X				X		X				X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma							X							X			X				X	X	X	X	X
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																									
C-cell adenoma							X								X		X						X	X	
Parathyroid	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	+	+	+
Adenoma, NOS																									
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
Islet cell carcinoma																		X							
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	N	N	+	N	N	+	N	N	N	N	N	N	+	+	N	+	+	+	+	+	+	+
Fibroadenoma																									
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor			X	X	X	X	X				X	X	X		X	X	X	X	X	X	X	X	X	X	X
Prostate	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																							X		
Adenoma, NOS				X													X	X			X				X
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Lacrimal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, malignant																				X					
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, metastatic																				X					
Leukemia, mononuclear cell				X		X	X	X				X	X		X	X		X				X		X	X

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 0 6	0 1 8	0 4 2	0 5 1	0 5 5	0 5 6	0 6 0	0 6 2	0 6 4	0 6 7	0 6 8	0 6 8	0 7 1	0 7 2	0 7 2	0 7 4	0 7 6	0 7 8	0 8 2	0 8 4	0 8 8	0 9 2	0 9 7	0 9 9	1 0 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 1	1 0 1	1 0 1	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Keratoacanthoma	X																									1
Subcutaneous tissue	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma									X												X					4
Fibrosarcoma			X																							1
Lipoma																						X				1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																						X				3
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma, NOS																										1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Squamous cell papilloma																		X								1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule				X					X										X							3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenocarcinoma									X																	1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	49
Adenoma, NOS				X		X			X			X			X						X		X			17
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma		X		X						X	X		X	X	X	X	X	X		X			X		X	21
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma						X																				1
C-cell adenoma							X				X			X				X							X	11
Parathyroid	+	+	+	–	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	41
Adenoma, NOS																X										1
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma														X												1
Islet cell carcinoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	M	M	+	M	+	M	M	M	+	+	M	+	M	+	+	M	M	+	+	+	+	+	M	+	+	*50
Fibroadenoma			X											X												2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	43
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Preputial/clitoral gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Carcinoma, NOS																							X			2
Adenoma, NOS							X														X					7
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Lacrimal gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Squamous cell carcinoma																								X		1
Zymbal gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	*50
Carcinoma, NOS																							X			1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, malignant																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Mesothelioma, metastatic																										1
Leukemia, mononuclear cell	X	X		X					X			X	X				X		X	X	X		X		X	24

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE: LOW DOSE

ANIMAL NUMBER	040	047	048	041	049	045	031	038	039	046	032	042	044	033	034	035	036	037	043	050	051	052	053	054	055
WEEKS ON STUDY	055	072	079	081	084	085	086	088	089	094	096	096	096	098	101	101	101	101	101	103	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Basal cell tumor																									
Trichoepithelioma																									
Sebaceous adenoma																									
Keratoacanthoma				X																					
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Fibroma																									
Fibrosarcoma				X																					
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	–	–	+	–	+	–	–	–	+	–	–	–	–	–	–	–
Trachea	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+	–	+	–	–	–	+	
Mesothelioma, NOS																		X							
Lymph nodes	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	+	+	–	–	+	–	–	–	–	
Thymus	+	+	+	+	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Squamous cell carcinoma, unclear primary/metas											X														
Liver	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	–	+	–	+	–	–	–	–	–	–
Hepatocellular carcinoma								X																	
Bile duct	+	+	+	+	+	+	+	+	+	–	–	+	+	+	–	–	+	–	+	–	–	–	–	–	–
Pancreas	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
Esophagus	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Squamous cell papilloma							X																		
Small intestine	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Large intestine	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Urinary bladder	–	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	–	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–
Carcinoma, NOS																									
Adenoma, NOS			X							X								X						
Adrenal	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–
Pheochromocytoma	X					X	X		X										X						
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Follicular cell adenoma																				X					
C-cell adenoma																									
C-cell carcinoma																									
Parathyroid	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	
REPRODUCTIVE SYSTEM																									
Mammary gland	M	M	+	M	M	M	+	M	M	M	+	M	M	M	M	M	M	M	M	+	M	M	M	M	M
Adenocarcinoma, NOS																				X					
Fibroadenoma																									
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mesothelioma, NOS																		X							
Prostate	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Preputial/clitoral gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Carcinoma, NOS																X		X							X
Adenoma, NOS																	X		X			X			
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																X									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Fibrosarcoma, metastatic				X																					
Leukemia, mononuclear cell			X		X	X	X	X	X	X		X	X	X	X	X	X		X		X				

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE
(Continued)

ANIMAL NUMBER	0 0 1 4	0 0 1 5	0 0 1 7	0 0 1 8	0 0 2 1	0 0 2 4	0 0 2 5	0 0 2 8	0 0 3 1	0 0 3 2	0 0 3 4	0 0 3 5	0 0 3 8	0 0 4 0	0 0 4 2	0 0 4 3	0 0 4 4	0 0 4 6	0 0 4 7	0 0 4 8	0 0 4 9	0 0 5 0	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	1 0 4	
INTEGUMENTARY SYSTEM																							
Skin	M	M	+	+	M	+	+	M	+	M	M	M	M	M	M	+	M	+	M	M	M	M	M	*50
Basal cell tumor									X															1
Trichoepithelioma							X																	1
Sebaceous adenoma						X																		1
Keratoacanthoma																		X						2
Subcutaneous tissue	M	M	M	+	M	+	+	M	+	M	M	M	M	M	M	+	M	+	M	M	M	M	M	*50
Fibroma																X								1
Fibrosarcoma				X																				1
RESPIRATORY SYSTEM																							
Lungs and bronchi	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	12
Trachea	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	8
HEMATOPOIETIC SYSTEM																							
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Spleen	–	–	–	–	–	–	–	–	+	+	–	+	–	–	–	+	–	–	–	+	+	–		26
Mesothelioma, NOS																								1
Lymph nodes	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	17
Thymus	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–		10
CIRCULATORY SYSTEM																							
Heart	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+		12
DIGESTIVE SYSTEM																							
Salivary gland	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11
Squamous cell carcinoma, ear pinna/meatus																								1
Liver	+	–	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	–	–	+	+	–		23
Hepatocellular carcinoma																								1
Bile duct	+	–	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	+	–	–	+	–		23
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–		11
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		9
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		8
Squamous cell papilloma																								1
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		8
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		8
URINARY SYSTEM																							
Kidney	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–		12
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		10
ENDOCRINE SYSTEM																							
Pituitary	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–		15
Carcinoma, NOS	X																							1
Adenoma, NOS						X			X											X				6
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–		13
Pheochromocytoma						X																		6
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Follicular cell adenoma																	X							1
C-cell adenoma																X					X			3
C-cell carcinoma																X		X						2
Parathyroid	+	+			+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+		44
REPRODUCTIVE SYSTEM																							
Mammary gland	M	M	M	M	M	M	+	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Adenocarcinoma, NOS																								1
Fibroadenoma									X															1
Testis	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Interstitial cell tumor	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		47
Mesothelioma, NOS																								1
Prostate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		10
Preputial/clitoral gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M		*50
Carcinoma, NOS																								3
Adenoma, NOS				X																				4
NERVOUS SYSTEM																							
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		2
BODY CAVITIES																							
Tunica vaginalis	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		*50
Mesothelioma, NOS																								1
ALL OTHER SYSTEMS																							
Multiple organs, NOS	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	*50
Fibrosarcoma, metastatic																								1
Leukemia, mononuclear cell									X			X				V				X				20

* Animals necropsied

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE: HIGH DOSE

ANIMAL NUMBER	042	004	017	041	026	001	037	042	028	023	048	031	019	021	034	020	009	010	027	044	002	003	006	011	012
WEEKS ON STUDY	052	072	079	081	083	084	085	085	086	090	090	092	092	093	094	098	101	103	103	103	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma															X										
Subcutaneous tissue	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myxosarcoma																								X	
Lipoma																									
Neurofibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	–	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Hepatocellular carcinoma															X										
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS							X		X			X	X			X		X	X	X				X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma			X	X				X				X			X		X			X	X	X	X	X	X
Pheochromocytoma, malignant																									
Thyroid	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma				X						X	X														
C-cell adenoma																			X	X	X				
C-cell carcinoma								X				X													
Parathyroid	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+
Adenoma, NOS											X				X										
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma					X						X														
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	N	N	+	+	N	N	+	N	+	+	+	+	+	+	N	+	+	+	+	N	N
Adenocarcinoma, NOS																									
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mesothelioma, NOS																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
Adenoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Zymbal gland	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS		X																							
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma															X										
Osteoma																									
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Myxosarcoma, invasive																								X	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell					X	X	X	X			X	X			X	X	X			X				X	X

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	013	014	015	016	018	022	024	025	028	029	030	031	032	033	035	036	037	038	039	040	043	045	046	047	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma																										1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Myxosarcoma																										1
Lipoma				X																						1
Neurofibrosarcoma									X																	1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																								1
Alveolar/bronchiolar carcinoma																								X		1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	44
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule											X	X														2
Hepatocellular carcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinar cell adenoma															X											1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma, NOS		X									X	X	X			X				X				X		16
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X		X					X	X			X	X		X			X	X	X	X	X				24
Pheochromocytoma, malignant								X																		1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell carcinoma		X																								4
C-cell adenoma						X		X							X									X		7
C-cell carcinoma																										2
Parathyroid	+	–	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	44
Adenoma, NOS			X		X																					4
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma			X																							3
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	N	+	N	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	*50
Adenocarcinoma, NOS														X												1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	48
Mesothelioma, NOS																					X					1
Prostate	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS															X											1
Adenoma, NOS	X		X		X																	X				4
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	*50
Squamous cell carcinoma																										1
Osteoma																									X	1
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Myxosarcoma, invasive																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell		X	X	X			X		X	X	X	X		X	X			X				X			X	25

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control	38 ppm	75 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	13.5%	3.2%	0.0%
Terminal Rates (c)	2/22 (9%)	1/31 (3%)	0/30 (0%)
Week of First Observation	83	104	
Life Table Tests (d)	P=0.014N	P=0.116N	P=0.043N
Incidental Tumor Tests (d)	P=0.024N	P=0.192N	P=0.058N
Cochran-Armitage Trend Test (d)	P=0.026N		
Fisher Exact Test (d)		P=0.181N	P=0.059N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	5/50 (10%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	16.8%	8.4%	0.0%
Terminal Rates (c)	2/22 (9%)	2/31 (6%)	0/30 (0%)
Week of First Observation	83	81	
Life Table Tests (d)	P=0.012N	P=0.238N	P=0.020N
Incidental Tumor Tests (d)	P=0.024N	P=0.399N	P=0.037N
Cochran-Armitage Trend Test (d)	P=0.023N		
Fisher Exact Test (d)		P=0.357N	P=0.028N
Subcutaneous Tissue: Fibroma, Fibrosarcoma, Neurofibrosarcoma, or Myxosarcoma			
Overall Rates (a)	5/50 (10%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	16.8%	8.4%	6.7%
Terminal Rates (c)	2/22 (9%)	2/31 (6%)	2/30 (7%)
Week of First Observation	83	81	104
Life Table Tests (d)	P=0.094N	P=0.238N	P=0.135N
Incidental Tumor Tests (d)	P=0.142N	P=0.399N	P=0.195N
Cochran-Armitage Trend Test (d)	P=0.158N		
Fisher Exact Test (d)		P=0.357N	P=0.218N
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	24/50 (48%)	(e) 20/50 (40%)	25/50 (50%)
Adjusted Rates (b)	64.3%		61.4%
Terminal Rates (c)	10/22 (45%)		15/30 (50%)
Week of First Observation	74		83
Life Table Test (d)			P=0.281N
Incidental Tumor Test (d)			P=0.520
Fisher Exact Test (d)			P=0.500
Liver: Neoplastic Nodule			
Overall Rates (a)	3/50 (6%)	(f) 0/23 (0%)	2/50 (4%)
Adjusted Rates (b)	13.6%		6.7%
Terminal Rates (c)	3/22 (14%)		2/30 (7%)
Week of First Observation	104		104
Life Table Test (d)			P=0.358N
Incidental Tumor Test (d)			P=0.358N
Fisher Exact Test (d)			P=0.500N
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	3/50 (6%)	(f) 1/23 (4%)	3/50 (6%)
Adjusted Rates (b)	13.6%		9.3%
Terminal Rates (c)	3/22 (14%)		2/30 (7%)
Week of First Observation	104		94
Life Table Test (d)			P=0.529N
Incidental Tumor Test (d)			P=0.569N
Fisher Exact Test (d)			P=0.661N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	38 ppm	75 ppm
Pituitary Gland: Adenoma			
Overall Rates (a)	17/49 (35%)	(f,g) 7/15 (40%)	16/50 (32%)
Adjusted Rates (b)	48.4%		41.4%
Terminal Rates (c)	7/21 (33%)		8/30 (27%)
Week of First Observation	53		85
Life Table Test (d)			P=0.232N
Incidental Tumor Test (d)			P=0.524N
Fisher Exact Test (d)			P=0.472N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	21/50 (42%)	(f) 6/13 (46%)	24/50 (48%)
Adjusted Rates (b)	66.4%		63.8%
Terminal Rates (c)	12/22 (55%)		17/30 (57%)
Week of First Observation	81		79
Life Table Test (d)			P=0.373N
Incidental Tumor Test (d)			P=0.503
Fisher Exact Test (d)			P=0.344
Thyroid Gland: Follicular Cell Carcinoma			
Overall Rates (a)	1/50 (2%)	0/49 (0%)	4/49 (8%)
Adjusted Rates (b)	4.5%	0.0%	10.0%
Terminal Rates (c)	1/22 (5%)	0/31 (0%)	1/30 (3%)
Week of First Observation	104		81
Life Table Tests (d)	P=0.109	P=0.432N	P=0.239
Incidental Tumor Tests (d)	P=0.082	P=0.432N	P=0.170
Cochran-Armitage Trend Test (d)	P=0.082		
Fisher Exact Test (d)		P=0.505N	P=0.175
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	1/50 (2%)	1/49 (2%)	4/49 (8%)
Adjusted Rates (b)	4.5%	3.2%	10.0%
Terminal Rates (c)	1/22 (5%)	1/31 (3%)	1/30 (3%)
Week of First Observation	104	104	81
Life Table Tests (d)	P=0.139	P=0.684N	P=0.239
Incidental Tumor Tests (d)	P=0.107	P=0.684N	P=0.170
Cochran-Armitage Trend Test (d)	P=0.099		
Fisher Exact Test (d)		P=0.747	P=0.175
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	11/50 (22%)	3/49 (6%)	7/49 (14%)
Adjusted Rates (b)	37.6%	9.7%	21.7%
Terminal Rates (c)	6/22 (27%)	3/31 (10%)	5/30 (17%)
Week of First Observation	81	104	103
Life Table Tests (d)	P=0.059N	P=0.005N	P=0.085N
Incidental Tumor Tests (d)	P=0.112N	P=0.015N	P=0.170N
Cochran-Armitage Trend Test (d)	P=0.163N		
Fisher Exact Test (d)		P=0.022N	P=0.232N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	11/50 (22%)	4/49 (8%)	9/49 (18%)
Adjusted Rates (b)	37.6%	12.9%	25.5%
Terminal Rates (c)	6/22 (27%)	4/31 (13%)	5/30 (17%)
Week of First Observation	81	104	85
Life Table Tests (d)	P=0.169N	P=0.012N	P=0.202N
Incidental Tumor Tests (d)	P=0.286N	P=0.030N	P=0.364N
Cochran-Armitage Trend Test (d)	P=0.353N		
Fisher Exact Test (d)		P=0.049N	P=0.421N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	38 ppm	75 ppm
Parathyroid: Adenoma			
Overall Rates (a)	1/41 (2%)	0/44 (0%)	4/44 (9%)
Adjusted Rates (b)	5.6%	0.0%	12.4%
Terminal Rates (c)	1/18 (6%)	0/27 (0%)	2/26 (8%)
Week of First Observation	104		90
Life Table Tests (d)	P=0.119	P=0.419N	P=0.269
Incidental Tumor Tests (d)	P=0.099	P=0.419N	P=0.219
Cochran-Armitage Trend Test (d)	P=0.093		
Fisher Exact Test (d)		P=0.482N	P=0.203
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	1/49 (2%)	(f) 0/11 (0%)	3/50 (6%)
Adjusted Rates (b)	4.5%		7.7%
Terminal Rates (c)	1/22 (5%)		1/30 (3%)
Week of First Observation	104		83
Life Table Test (d)			P=0.382
Incidental Tumor Test (d)			P=0.330
Fisher Exact Test (d)			P=0.316
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	2/49 (4%)	(f) 0/11 (0%)	3/50 (6%)
Adjusted Rates (b)	7.3%		7.7%
Terminal Rates (c)	1/22 (5%)		1/30 (3%)
Week of First Observation	92		83
Life Table Test (d)			P=0.582
Incidental Tumor Test (d)			P=0.511
Fisher Exact Test (d)			P=0.510
Preputial Gland: Adenoma			
Overall Rates (a)	7/50 (14%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	21.9%	12.2%	13.3%
Terminal Rates (c)	2/22 (9%)	3/31 (10%)	4/30 (13%)
Week of First Observation	74	101	104
Life Table Tests (d)	P=0.109N	P=0.143N	P=0.157N
Incidental Tumor Tests (d)	P=0.191N	P=0.277N	P=0.263N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.262N	P=0.262N
Preputial Gland: Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	8.0%	8.6%	3.3%
Terminal Rates (c)	1/22 (5%)	1/31 (3%)	1/30 (3%)
Week of First Observation	99	101	104
Life Table Tests (d)	P=0.300N	P=0.640	P=0.406N
Incidental Tumor Tests (d)	P=0.407N	P=0.563	P=0.469N
Cochran-Armitage Trend Test (d)	P=0.403N		
Fisher Exact Test (d)		P=0.500	P=0.500N
Preputial Gland: Adenoma or Carcinoma			
Overall Rates (a)	9/50 (18%)	7/50 (14%)	5/50 (10%)
Adjusted Rates (b)	28.4%	20.2%	16.7%
Terminal Rates (c)	3/22 (14%)	4/31 (13%)	5/30 (17%)
Week of First Observation	74	101	104
Life Table Tests (d)	P=0.068N	P=0.200N	P=0.097N
Incidental Tumor Tests (d)	P=0.148N	P=0.371N	P=0.184N
Cochran-Armitage Trend Test (d)	P=0.157N		
Fisher Exact Test (d)		P=0.393N	P=0.194N

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	38 ppm	75 ppm
Testis: Interstitial Cell Tumor			
Overall Rates (a)	43/50 (86%)	47/49 (96%)	48/50 (96%)
Adjusted Rates (b)	97.7%	100.0%	100.0%
Terminal Rates (c)	21/22 (95%)	30/30 (100%)	30/30 (100%)
Weeks to First Observation	67	72	72
Life Table Tests (d)	P=0.199N	P=0.145N	P=0.231N
Incidental Tumor Tests (d)	P=0.109	P=0.274	P=0.162
Cochran-Armitage Trend Test (d)	P=0.042		
Fisher Exact Test (d)		P=0.084	P=0.080

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. N indicates a negative trend or lower incidence in a dosed group.
(e) Only 23 livers, 26 spleens, and 17 lymph nodes were examined.
(f) All animals were examined grossly at the site, and lesions found were evaluated microscopically. The incidence listed represents the number of animals with lesions diagnosed as tumors divided by the number of animals with gross lesions.
(g) Includes one carcinoma, NOS

TABLE A4. HISTORICAL INCIDENCE OF PARATHYROID ADENOMAS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

	Incidence in Controls
Historical Incidence at Battelle Columbus Laboratories	
Chlorobenzene	0/41
Pooled control group (b)	0/70
C.I. Disperse Yellow 3	0/34
D & C Red 9	1/41
C.I. Solvent Yellow 14	0/38
L-Ascorbic acid	0/37
TOTAL	1/261 (0.4%)
SD (c)	1.09%
Range (d)	
High	1/41
Low	0/70
Overall Historical Incidence	
TOTAL	(e) 4/1,314 (0.3%)
SD (c)	0.76%
Range (d)	
High	1/38
Low	0/70

(a) Data as of August 30, 1985, for studies of at least 104 weeks; no malignant parathyroid tumors have been observed in untreated control groups.
(b) Common control group for C.I. Acid Orange 10, FD & C Yellow No. 6, and C.I. Acid Red 14
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) The mean of the individual incidences is 0.4%.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Epidermal inclusion cyst	1 (2%)		1 (2%)
Inflammation, acute/chronic		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
Inflammation, granulomatous focal	1 (2%)		
RESPIRATORY SYSTEM			
*Nasal mucosa	(50)	(50)	(50)
Inflammation, acute focal	18 (36%)		18 (36%)
Inflammation, acute diffuse	1 (2%)		1 (2%)
Inflammation, acute necrotizing			1 (2%)
Inflammation, chronic focal	3 (6%)		1 (2%)
Necrosis, diffuse		1 (2%)	
Hyperplasia, epithelial	1 (2%)		
*Nasal turbinate	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
#Lung	(50)	(12)	(50)
Mineralization			1 (2%)
Congestion, acute			1 (2%)
Inflammation, acute suppurative			2 (4%)
Pneumonia, interstitial chronic	2 (4%)		4 (8%)
Hyperplasia, epithelial		1 (8%)	2 (4%)
Metaplasia, osseous		1 (8%)	
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(9)	(50)
Myelofibrosis	2 (4%)		5 (10%)
Hyperplasia, granulocytic	2 (4%)		
Hypoplasia, hematopoietic			1 (2%)
#Spleen	(49)	(26)	(50)
Necrosis, focal		1 (4%)	1 (2%)
#Splenic capsule	(49)	(26)	(50)
Cyst, NOS	1 (2%)		
Fibrosis, multifocal			1 (2%)
#Splenic follicles	(49)	(26)	(50)
Depletion, lymphoid	1 (2%)	2 (8%)	1 (2%)
#Splenic red pulp	(49)	(26)	(50)
Fibrosis, focal	3 (6%)	1 (4%)	4 (8%)
Fibrosis, multifocal	7 (14%)	3 (12%)	2 (4%)
Pigmentation, NOS			2 (4%)
Hematopoiesis	2 (4%)	1 (4%)	1 (2%)
#Mandibular lymph node	(50)	(17)	(50)
Hemorrhage	1 (2%)	1 (6%)	
Inflammation, acute focal			1 (2%)
Inflammation, acute/chronic			1 (2%)
Inflammation, granulomatous focal	2 (4%)		2 (4%)
Depletion, lymphoid		1 (6%)	1 (2%)
#Bronchial lymph node	(50)	(17)	(50)
Hemorrhage		1 (6%)	
Depletion, lymphoid		1 (6%)	
#Mediastinal lymph node	(50)	(17)	(50)
Hemorrhage	3 (6%)	2 (12%)	4 (8%)
Inflammation, granulomatous focal	2 (4%)		2 (4%)
Depletion, lymphoid		2 (12%)	

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#Pancreatic lymph node	(50)	(17)	(50)
Inflammation, granulomatous focal			1 (2%)
#Lumbar lymph node	(50)	(17)	(50)
Plasmacytosis	1 (2%)		
#Mesenteric lymph node	(50)	(17)	(50)
Hemorrhage		1 (6%)	
Inflammation, granulomatous focal	1 (2%)		1 (2%)
Depletion, lymphoid		1 (6%)	
#Inguinal lymph node	(50)	(17)	(50)
Inflammation, granulomatous focal			1 (2%)
Plasmacytosis	1 (2%)		
#Thymic lymph node	(50)	(17)	(50)
Inflammation, granulomatous focal	1 (2%)		
#Thymus	(41)	(10)	(44)
Embryonal duct cyst	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
#Thymic cortex	(41)	(10)	(44)
Depletion, lymphoid	21 (51%)	2 (20%)	22 (50%)
CIRCULATORY SYSTEM			
#Heart/atrium	(50)	(12)	(50)
Thrombosis, NOS	1 (2%)	2 (17%)	4 (8%)
Inflammation, acute/chronic	1 (2%)		
#Myocardium	(50)	(12)	(50)
Degeneration, NOS	41 (82%)	10 (83%)	43 (86%)
#Papillary muscle of conus	(50)	(12)	(50)
Metaplasia, cartilaginous			1 (2%)
#Aortic valve	(50)	(12)	(50)
Degeneration, NOS	2 (4%)		
*Aorta	(50)	(50)	(50)
Mineralization			1 (2%)
Inflammation, acute focal	1 (2%)		
Inflammation, active chronic	1 (2%)		
*Pulmonary artery	(50)	(50)	(50)
Mineralization	1 (2%)		
*Mediastinal artery	(50)	(50)	(50)
Inflammation, active chronic	1 (2%)		
*Sup. pancreaticoduodenal artery	(50)	(50)	(50)
Mineralization	1 (2%)		
Inflammation, active chronic	7 (14%)		8 (16%)
DIGESTIVE SYSTEM			
#Liver	(50)	(23)	(50)
Congestion, NOS	1 (2%)		
Inflammation, granulomatous focal	4 (8%)		3 (6%)
Degeneration, cystic	12 (24%)	4 (17%)	21 (42%)
Necrosis, focal	3 (6%)	1 (4%)	2 (4%)
Basophilic cyto change	20 (40%)	4 (17%)	32 (64%)
Eosinophilic cyto change	2 (4%)		6 (12%)
Clear cell change			5 (10%)
Angiectasis	4 (8%)	4 (17%)	8 (16%)
#Liver/centrilobular	(50)	(23)	(50)
Degeneration, NOS			1 (2%)
#Liver/hepatocytes	(50)	(23)	(50)
Necrosis, diffuse	1 (2%)		
Cytoplasmic vacuolization	9 (18%)		9 (18%)
#Bile duct	(50)	(23)	(50)
Cyst, NOS		1 (4%)	
Hyperplasia, focal	42 (84%)	15 (65%)	30 (60%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#Pancreas	(49)	(11)	(50)
Inflammation, acute diffuse	1 (2%)		
Focal cellular change	1 (2%)		
#Pancreatic acinus	(49)	(11)	(50)
Atrophy, focal	12 (24%)	7 (64%)	14 (28%)
Hyperplasia, focal	1 (2%)		
#Esophagus	(49)	(9)	(49)
Inflammation, acute necrotizing	1 (2%)		
Inflammation, acute/chronic			1 (2%)
#Glandular stomach	(49)	(8)	(49)
Mineralization	1 (2%)		1 (2%)
Ulcer, NOS	2 (4%)		
Inflammation, acute focal	5 (10%)		10 (20%)
Inflammation, chronic focal	1 (2%)		
Necrosis, focal	2 (4%)	1 (13%)	1 (2%)
#Forestomach	(49)	(8)	(49)
Ulcer, NOS	2 (4%)	1 (13%)	1 (2%)
Inflammation, acute focal	3 (6%)		1 (2%)
Inflammation, acute/chronic	1 (2%)		2 (4%)
Inflammation, chronic focal	2 (4%)		
Hyperkeratosis			1 (2%)
Acanthosis	1 (2%)		1 (2%)
#Duodenum	(49)	(8)	(49)
Inflammation, acute diffuse	1 (2%)		
Necrosis, focal			1 (2%)
#Jejunum	(49)	(8)	(49)
Inflammation, acute diffuse	1 (2%)		
Granuloma, NOS	1 (2%)		
#Colon	(50)	(8)	(49)
Edema, NOS			1 (2%)
Inflammation, acute diffuse	1 (2%)		1 (2%)
Parasitism	5 (10%)	1 (13%)	2 (4%)
#Cecum	(50)	(8)	(49)
Edema, NOS			1 (2%)
Inflammation, acute focal			1 (2%)
Necrosis, focal			1 (2%)
*Rectum	(50)	(50)	(50)
Parasitism	2 (4%)		2 (4%)
URINARY SYSTEM			
#Urinary bladder/cavity	(48)	(10)	(49)
Dilatation, NOS	1 (2%)		
#Kidney	(50)	(12)	(50)
Hydronephrosis			1 (2%)
Pyelonephritis, acute	1 (2%)		
Inflammation, acute/chronic			1 (2%)
Nephropathy	50 (100%)	11 (92%)	48 (96%)
Infarct, acute	1 (2%)		
#Kidney/cortex	(50)	(12)	(50)
Cyst, NOS	2 (4%)	1 (8%)	2 (4%)
#Kidney/pelvis	(50)	(12)	(50)
Hyperplasia, epithelial	8 (16%)		4 (8%)
#Urinary bladder	(48)	(10)	(49)
Hemorrhage	1 (2%)		
Inflammation, acute necrotizing	1 (2%)		
Hyperplasia, epithelial	3 (6%)		2 (4%)

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(15)	(50)
Multiple cysts		1 (7%)	
#Anterior pituitary	(49)	(15)	(50)
Cyst, NOS	4 (8%)		4 (8%)
Multiple cysts	2 (4%)		3 (6%)
Hemorrhage	1 (2%)		
Inflammation, chronic focal			1 (2%)
Cytoplasmic vacuolization			1 (2%)
Focal cellular change		1 (7%)	1 (2%)
Hyperplasia, focal	7 (14%)	2 (13%)	13 (26%)
#Adrenal	(50)	(13)	(50)
Cyst, NOS		1 (8%)	
#Adrenal/capsule	(50)	(13)	(50)
Ectopia	1 (2%)		
#Adrenal cortex	(50)	(13)	(50)
Necrosis, focal			1 (2%)
Metamorphosis, fatty	12 (24%)	2 (15%)	9 (18%)
Focal cellular change	5 (10%)	1 (8%)	1 (2%)
Atrophy, diffuse			1 (2%)
Hypertrophy, focal	1 (2%)		
Hyperplasia, focal	7 (14%)		9 (18%)
#Adrenal medulla	(50)	(13)	(50)
Cyst, NOS			1 (2%)
Hyperplasia, focal	18 (36%)	1 (8%)	16 (32%)
#Thyroid	(50)	(49)	(49)
Follicular cyst, NOS	4 (8%)		5 (10%)
Inflammation, acute/chronic			1 (2%)
Hyperplasia, C-cell	23 (46%)	38 (78%)	37 (76%)
Hyperplasia, follicular cell			1 (2%)
#Parathyroid	(41)	(44)	(44)
Inflammation, acute/chronic			1 (2%)
Focal cellular change			2 (5%)
Hyperplasia, NOS	1 (2%)	1 (2%)	4 (9%)
Hyperplasia, focal	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Hyperplasia, cystic	23 (46%)	1 (2%)	27 (54%)
*Preputial gland	(50)	(50)	(50)
Dilatation/ducts	1 (2%)		1 (2%)
Cyst, NOS	2 (4%)		1 (2%)
Abscess, NOS	2 (4%)		
Inflammation, granulomatous focal	30 (60%)	3 (6%)	41 (82%)
Hyperplasia, focal	4 (8%)		6 (12%)
#Prostate	(48)	(10)	(49)
Inflammation, acute/chronic	15 (31%)	2 (20%)	17 (35%)
Abscess, chronic	1 (2%)		
*Seminal vesicle	(50)	(50)	(50)
Abscess, chronic	1 (2%)		
Fibrosis, multifocal		1 (2%)	
#Testis	(50)	(49)	(50)
Inflammation, acute/chronic	1 (2%)		
Necrosis, diffuse	1 (2%)		1 (2%)
Atrophy, NOS	1 (2%)		1 (2%)
Hyperplasia, interstitial cell	20 (40%)	3 (6%)	11 (22%)
*Epididymis	(50)	(50)	(50)
Dilatation, NOS	1 (2%)		
Inflammation, chronic focal	1 (2%)		

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#Cerebral ventricle	(50)	(9)	(50)
Hydrocephalus, NOS	2 (4%)		1 (2%)
#Brain	(50)	(9)	(50)
Mineralization	1 (2%)		
Hemorrhage	5 (10%)		3 (6%)
Necrosis, focal	1 (2%)		
Atrophy, pressure	2 (4%)	1 (11%)	1 (2%)
SPECIAL SENSE ORGANS			
*Eye/anterior chamber	(50)	(50)	(50)
Hemorrhage			1 (2%)
*Eye/cornea	(50)	(50)	(50)
Ulcer, NOS			1 (2%)
Inflammation, acute focal			1 (2%)
*Eye/retina	(50)	(50)	(50)
Atrophy, focal			2 (4%)
*Eye/crystalline lens	(50)	(50)	(50)
Cataract			2 (4%)
*Nasolacrimal duct	(50)	(50)	(50)
Inflammation, acute focal	1 (2%)		
*Harderian gland	(50)	(50)	(50)
Inflammation, necrotizing			1 (2%)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, focal			1 (2%)
Regeneration, NOS	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
*Cortex of bone	(50)	(50)	(50)
Hyperplasia, focal	2 (4%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
*Epicardium	(50)	(50)	(50)
Inflammation, acute focal	1 (2%)		
*Mesentery	(50)	(50)	(50)
Hemorrhage, chronic	1 (2%)		
Inflammation, multifocal			1 (2%)
Inflammation, granulomatous focal			1 (2%)
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

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APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS

IN THE TWO-YEAR FEED STUDY OF ROTENONE

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	46	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell papilloma		1 (2%)	
Keratoacanthoma		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS		1 (2%)	1 (2%)
Fibroma		1 (2%)	
Fibrosarcoma		1 (2%)	2 (4%)
Fibrous histiocytoma, malignant		1 (2%)	
Myxosarcoma		1 (2%)	
Neurofibroma		1 (2%)	
Neurilemoma, malignant			1 (2%)
RESPIRATORY SYSTEM			
#Peritracheal tissue	(50)	(9)	(50)
Paraganglioma, NOS		1 (11%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	15 (30%)	10 (20%)	13 (26%)
#Spleen	(50)	(19)	(50)
Leukemia, mononuclear cell		2 (11%)	
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)		
#Salivary gland	(50)	(10)	(49)
Sarcoma, NOS, invasive		1 (10%)	
#Liver	(50)	(19)	(50)
Neoplastic nodule	1 (2%)		
#Pancreas	(49)	(9)	(49)
Acinar cell adenoma	1 (2%)		
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Anterior pituitary	(50)	(30)	(49)
Carcinoma, NOS	4 (8%)	1 (3%)	1 (2%)
Adenoma, NOS	25 (50%)	15 (50%)	22 (45%)
#Adrenal	(50)	(11)	(48)
Cortical adenoma			2 (4%)
#Adrenal medulla	(50)	(11)	(48)
Pheochromocytoma	4 (8%)	1 (9%)	4 (8%)
Pheochromocytoma, malignant		1 (9%)	

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(11)	(47)
C-cell adenoma	9 (18%)	1 (9%)	9 (19%)
C-cell carcinoma		2 (18%)	
#Parathyroid	(42)	(7)	(41)
Adenoma, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenoma, NOS	1 (2%)	3 (6%)	2 (4%)
Adenocarcinoma, NOS	1 (2%)	1 (2%)	
Fibroadenoma	11 (22%)	10 (20%)	10 (20%)
*Clitoral gland	(50)	(50)	(50)
Carcinoma, NOS	1 (2%)		1 (2%)
Adenoma, NOS	2 (4%)	1 (2%)	1 (2%)
#Uterus	(50)	(17)	(50)
Endometrial stromal polyp	5 (10%)	5 (29%)	6 (12%)
#Cervix uteri	(50)	(17)	(50)
Endometrial stromal polyp		1 (6%)	
#Ovary	(50)	(13)	(50)
Granulosa cell tumor	1 (2%)	1 (8%)	
NERVOUS SYSTEM			
#Cerebrum	(50)	(9)	(50)
Carcinoma, NOS, invasive	3 (6%)		
Astrocytoma	1 (2%)		1 (2%)
SPECIAL SENSE ORGANS			
*Zymbal gland	(50)	(50)	(50)
Carcinoma, NOS	2 (4%)	1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	8	5	4
Moribund sacrifice	15	13	15
Terminal sacrifice	27	32	31

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
Total animals with primary tumors**	45	43	45
Total primary tumors	85	65	78
Total animals with benign tumors	38	35	39
Total benign tumors	58	41	57
Total animals with malignant tumors	24	19	20
Total malignant tumors	25	22	21
Total animals with secondary tumors##	3	1	
Total secondary tumors	3	1	
Total animals with tumors uncertain--benign or malignant	2	2	
Total uncertain tumors	2	2	

* Number of animals receiving complete necropsy examination: all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE: UNTREATED CONTROL

ANIMAL NUMBER	0 0 9	0 1 6	0 3 6	0 6 4	0 1 7	0 4 8	0 6 8	0 2 1	0 0 7	0 1 8	0 2 2	0 4 6	0 2 6	0 2 3	0 3 0	0 4 2	0 2 7	0 3 0	0 2 0	0 1 1	0 4 1	0 2 9	0 3 1	0 0 1	0 0 2
WEEKS ON STUDY	0 3 3	0 3 3	0 3 3	0 6 4	0 6 5	0 7 2	0 7 4	0 8 1	0 8 6	0 8 8	0 9 4	0 9 4	1 1 4	1 2 3	0 5 5	0 9 7	0 9 8	0 9 8	0 9 8	1 0 0	1 0 0	1 0 2	1 0 3	1 0 4	1 0 4
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
Thymus	–	+	+	–	–	+	–	–	+	+	+	+	–	+	+	+	–	+	–	–	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma																	X								
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS		X																							X
Adenoma, NOS			X				X	X	X	X	X		X	X	X	X					X	X	X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Pheochromocytoma																							X		
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell adenoma									X					X						X					
Parathyroid	+	+	+	–	+	+	–	–	–	+	–	–	+	+	+	+	+	+	+	–	–	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	N	+	N	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+
Adenoma, NOS		X																							
Adenocarcinoma, NOS								X																	
Fibroadenoma																	X						X		
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS															X										
Adenoma, NOS																									X
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp									X															X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive		X																							X
Astrocytoma						X																			
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	+	N	N	N	N	N	N	N	N
Carcinoma, NOS															X		X								
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell							X			X	X	X		X		X			X						

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 0 3	0 0 8	0 0 8	0 1 0	0 1 2	0 1 3	0 1 4	0 1 5	0 1 9	0 2 2	0 2 4	0 2 5	0 2 8	0 2 8	0 3 3	0 3 4	0 3 5	0 3 7	0 3 8	0 3 9	0 4 3	0 4 4	0 4 5	0 4 7	0 4 9	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 1	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	46
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule									X																	1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	49
Acinar cell adenoma																						X				1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS					X	X																				4
Adenoma, NOS			X				X	X	X	X	X		X	X						X	X	X	X	X		25
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma									X						X		X									4
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell adenoma											X						X	X	X			X	X			9
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenoma, NOS																										1
Adenocarcinoma, NOS																										1
Fibroadenoma		X						X	X			X	X	X				X				X			X	11
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Adenoma, NOS																								X		2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp					X						X							X								5
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor															X											1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, invasive						X																				3
Astrocytoma																										1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell							X			X		X	X			X		X	X					X		15

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE: LOW DOSE

ANIMAL NUMBER	041	067	036	048	028	019	039	040	004	030	018	046	005	042	049	037	008	016	001	002	003	006	007	009	010	011
WEEKS ON STUDY	064	067	071	081	082	084	085	085	091	093	096	096	097	099	099	100	101	103	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	N	N	N	N	N	+	+	N	N	+	N	N	N	N	N	N	N
Squamous cell papilloma																	X									
Keratoacanthoma																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	N	N	N	N	N	+	+	N	N	+	N	N	N	N	N	N	N
Sarcoma, NOS																										
Fibroma			X																							
Fibrosarcoma															X											
Fibrous histiocytoma, malignant				X																						
Myxosarcoma	X																									
Neurofibroma																			X							
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	C	–	–	C	
Trachea	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
Paraganglioma, NOS							X																			
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
Spleen	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	–	+	–	–	–	–	C	+	–	C	
Leukemia, mononuclear cell																										
Lymph nodes	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	–	C	–	–	C	
Thymus	+	–	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	C	–	–	C	
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
Sarcoma, NOS, invasive																										
Liver	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	+	+	–	–	–	–	C	–	–	C	
Bile duct	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	+	+	–	–	–	–	C	–	–	C	
Pancreas	+	+	+	+	–	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	C	–	–	C	
Esophagus	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
Stomach	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	C	–	–	C	
Small intestine	+	+	+	+	–	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	C	–	–	C	
Large intestine	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–	–	–	–	–	C	–	–	C	
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	C	–	–	C	
Urinary bladder	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	–	C	+	–	C	
Carcinoma, NOS																X										
Adenoma, NOS					X		X			X	X			X					X	X			X			
Adrenal	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	+	+	–	–	–	C	–	–	C	
Pheochromocytoma																		X								
Pheochromocytoma, malignant																X										
Thyroid	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	C	–	–	C	
C-cell adenoma									X																	
C-cell carcinoma													X													
Parathyroid	+	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	+	+	+	+	+	+	+	+	N	N	N	+	+	N	+	N	N	+	N	N	N	N	
Adenoma, NOS																X										
Adenocarcinoma, NOS																										
Fibroadenoma							X	X		X	X							X			X					
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																										
Uterus	+	+	+	+	+	+	+	+	+	–	–	+	+	–	–	–	+	–	–	–	+	C	–	+	C	
Endometrial stromal polyp		X		X		X																		X		
Ovary	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	+	C	
Granulosa cell tumor																								X		
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	C	–	–	C	
SPECIAL SENSE ORGANS																										
Zymbal gland	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	
Carcinoma, NOS		X																								
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell					X				X		X	X	X	X				X						X		

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	012	013	014	015	017	020	021	022	023	024	025	026	027	028	031	032	033	034	035	038	043	044	045	047	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	+	N	N	N	N	N	+	*50
Squamous cell papilloma											X															1
Keratoacanthoma																										1
Subcutaneous tissue	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	+	N	N	N	N	N	+	*50
Sarcoma, NOS																									X	1
Fibroma																										1
Fibrosarcoma																										1
Fibrous histiocytoma, malignant																										1
Myxosarcoma																										1
Neurofibroma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	+	–	–	+	–	–	–	–	12
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	9
Paraganglioma, NOS																										1
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	9
Spleen	–	–	–	–	+	+	–	–	–	–	+	–	–	C	–	C	–	+	–	–	–	–	–	–	–	19
Leukemia, mononuclear cell						X					X															2
Lymph nodes	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	11
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	8
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	9
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	+	10
Sarcoma, NOS, invasive																									X	1
Liver	–	–	+	–	+	–	–	+	–	+	–	–	–	C	–	C	–	+	–	–	–	–	–	–	–	19
Bile duct	–	–	+	–	+	–	–	+	–	+	–	–	–	C	–	C	–	+	–	–	–	–	–	–	–	19
Pancreas	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	6
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	9
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	10
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	9
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	10
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	10
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	8
ENDOCRINE SYSTEM																										
Pituitary	–	+	–	+	+	–	–	+	+	+	–	–	–	C	+	C	–	+	–	+	–	–	+	+	+	30
Carcinoma, NOS																										1
Adenoma, NOS				X	X			X		X					X					X			X		15
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	11
Pheochromocytoma																										1
Pheochromocytoma, malignant																										1
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	+	–	–	–	–	–	–	–	11
C-cell adenoma																										1
C-cell carcinoma																		X								2
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	7
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	N	N	N	N	+	N	+	N	+	N	N	N	N	N	N	+	+	+	N	+	N	N	N	*50
Adenoma, NOS		X							X																	3
Adenocarcinoma, NOS																						X				1
Fibroadenoma	X						X				X								X							10
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS			X																							1
Uterus	–	–	–	–	–	–	–	–	–	–	–	–	+	C	–	C	–	–	–	–	–	–	+	–	+	17
Endometrial stromal polyp																							X		X	6
Ovary	–	–	–	–	–	–	–	+	–	–	–	–	–	C	–	C	–	+	–	+	–	–	–	–	–	13
Granulosa cell tumor																										1
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	C	–	C	–	–	–	–	–	–	–	–	–	9
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell					X													X								10

* Animals necropsied

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE: HIGH DOSE

ANIMAL NUMBER	020	015	049	027	034	008	046	028	033	053	004	014	010	013	017	019	016	026	031	001	002	005	006	007	009
WEEKS ON STUDY	087	097	097	081	081	082	084	085	085	086	092	092	096	097	097	101	102	102	102	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																	X								
Fibrosarcoma			X						X																
Neurilemoma, malignant				X																					
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	−	+	−	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Carcinoma, NOS																						X			
Adenoma, NOS						X			X				X	X	X		X		X	X			X	X	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+
Cortical adenoma																									
Pheochromocytoma							X									X									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+
C-cell adenoma																					X	X			
Parathyroid	−	+	+	−	+	+	−	+	+	−	+	+	+	+	−	+	+	−	−	+	+	+	+	+	+
Adenoma, NOS																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Adenoma, NOS											X														
Fibroadenoma		X		X									X							X		X			
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS										X															
Adenoma, NOS																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp	X																								X
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma																									
SPECIAL SENSE ORGANS																									
Zymbal gland	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Carcinoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell	X						X	X			X		X	X	X	X		X				X			

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	0111	0114	0118	0121	0122	0123	0124	0125	0126	0130	0132	0135	0136	0137	0138	0139	0140	0141	0142	0143	0144	0145	0147	0148	0150	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																										1
Fibrosarcoma																										2
Neurilemoma, malignant																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	43
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																										1
Adenoma, NOS	X			X			X		X							X	X	X	X	X			X	X	X	23
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cortical adenoma									X					X												3
Pheochromocytoma	X	X																								4
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
C-cell adenoma			X	X	X		X	X		X										X						9
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+		41
Adenoma, NOS																								X		1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Adenoma, NOS		X																								2
Fibroadenoma			X			X													X				X		X	10
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS																										1
Adenoma, NOS						X																				1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp					X			X						X										X		6
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma		X																								1
SPECIAL SENSE ORGANS																										
Zymbal gland	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Carcinoma, NOS						X																				1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell																X	X				X					13

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control	38 ppm	75 ppm
Subcutaneous Tissue: Sarcoma, Fibrosarcoma, or Myxosarcoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	0.0%	7.6%	7.1%
Terminal Rates (c)	0/27 (0%)	1/32 (3%)	0/31 (0%)
Week of First Observation		64	77
Life Table Tests (d)	P=0.121	P=0.147	P=0.143
Incidental Tumor Tests (d)	P=0.057	P=0.075	P=0.091
Cochran-Armitage Trend Test (d)	P=0.100		
Fisher Exact Test (d)		P=0..21	P=0.121
Subcutaneous Tissue: Fibroma, Neurofibroma, Sarcoma, Fibrosarcoma, or Myxosarcoma			
Overall Rates (a)	0/50 (0%)	5/50 (10%)	3/50 (6%)
Adjusted Rates (b)	0.0%	12.5%	7.1%
Terminal Rates (c)	0/27 (0%)	2/32 (6%)	0/31 (0%)
Week of First Observation		64	77
Life Table Tests (d)	P=0.163	P=0.049	P=0.143
Incidental Tumor Tests (d)	P=0.067	P=0.013	P=0.091
Cochran-Armitage Trend Test (d)	P=0.131		
Fisher Exact Test (d)		P=0.028	P=0.121
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	15/50 (30%)	(e) 12/50 (24%)	13/50 (26%)
Adjusted Rates (b)	41.6%		31.0%
Terminal Rates (c)	8/27 (30%)		4/31 (13%)
Week of First Observation	74		67
Life Table Test (d)			P=0.327N
Incidental Tumor Test (d)			P=0.485N
Fisher Exact Test (d)			P=0.412N
Pituitary Gland: Adenoma			
Overall Rates (a)	25/50 (50%)	(f) 15/30 (50%)	22/49 (45%)
Adjusted Rates (b)	62.9%		58.6%
Terminal Rates (c)	13/27 (48%)		15/30 (50%)
Week of First Observation	64		83
Life Table Test (d)			P=0.243N
Incidental Tumor Test (d)			P=0.356N
Fisher Exact Test (d)			P=0.380N
Pituitary Gland: Carcinoma			
Overall Rates (a)	4/50 (8%)	(f) 1/30 (3%)	1/49 (2%)
Adjusted Rates (b)	12.9%		3.3%
Terminal Rates (c)	3/27 (11%)		1/30 (3%)
Week of First Observation	53		104
Life Table Test (d)			P=0.155N
Incidental Tumor Test (d)			P=0.192N
Fisher Exact Test (d)			P=0.187N
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	29/50 (58%)	(f) 16/30 (53%)	23/49 (47%)
Adjusted Rates (b)	71.4%		61.4%
Terminal Rates (c)	16/27 (59%)		16/30 (53%)
Week of First Observation	53		83
Life Table Test (d)			P=0.107N
Incidental Tumor Test (d)			P=0.165N
Fisher Exact Test (d)			P=0.184N

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	38 ppm	75 ppm
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	4/50 (8%)	(f,g) 2/11	4/48 (8%)
Adjusted Rates (b)	14.3%		11.2%
Terminal Rates (c)	3/27 (11%)		2/31 (6%)
Week of First Observation	103		84
Life Table Test (d)			P=0.570N
Incidental Tumor Test (d)			P=0.642
Fisher Exact Test (d)			P=0.619
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	9/50 (18%)	(f,h) 3/11 (27%)	9/47 (19%)
Adjusted Rates (b)	28.5%		29.0%
Terminal Rates (c)	6/27 (22%)		9/31 (29%)
Week of First Observation	86		104
Life Table Test (d)			P=0.490N
Incidental Tumor Test (d)			P=0.557N
Fisher Exact Test (d)			P=0.545
Mammary Gland: Adenoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	2.0%	8.9%	5.6%
Terminal Rates (c)	0/27 (0%)	2/32 (6%)	1/31 (3%)
Week of First Observation	53	100	92
Life Table Tests (d)	P=0.434	P=0.351	P=0.518
Incidental Tumor Tests (d)	P=0.399	P=0.245	P=0.558
Cochran-Armitage Trend Test (d)	P=0.398		
Fisher Exact Test (d)		P=0.309	P=0.500
Mammary Gland: Fibroadenoma			
Overall Rates (a)	11/50 (22%)	10/50 (20%)	10/50 (20%)
Adjusted Rates (b)	37.6%	25.7%	27.7%
Terminal Rates (c)	9/27 (33%)	5/32 (16%)	7/31 (23%)
Week of First Observation	98	85	77
Life Table Tests (d)	P=0.343N	P=0.356N	P=0.373N
Incidental Tumor Tests (d)	P=0.406N	P=0.407N	P=0.463N
Cochran-Armitage Trend Test (d)	P=0.451N		
Fisher Exact Test (d)		P=0.500N	P=0.500N
Mammary Gland: Adenoma or Fibroadenoma			
Overall Rates (a)	12/50 (24%)	13/50 (26%)	12/50 (24%)
Adjusted Rates (b)	38.9%	33.2%	32.5%
Terminal Rates (c)	9/27 (33%)	7/32 (22%)	8/31 (26%)
Week of First Observation	53	85	77
Life Table Tests (d)	P=0.426N	P=0.518N	P=0.464N
Incidental Tumor Tests (d)	P=0.506N	P=0.555	P=0.538N
Cochran-Armitage Trend Test (d)	P=0.546		
Fisher Exact Test (d)		P=0.500	P=0.592
Mammary Gland: Adenoma or Adenocarcinoma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	4.3%	12.0%	5.6%
Terminal Rates (c)	0/27 (0%)	3/32 (9%)	1/31 (3%)
Week of First Observation	53	100	92
Life Table Tests (d)	P=0.547N	P=0.397	P=0.670N
Incidental Tumor Tests (d)	P=0.525N	P=0.348	P=0.558N
Cochran-Armitage Trend Test (d)	P=0.586		
Fisher Exact Test (d)		P=0.339	P=0.691

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	38 ppm	75 ppm
Clitoral Gland: Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	9.9%	3.1%	5.6%
Terminal Rates (c)	2/27 (7%)	1/32 (3%)	1/31 (3%)
Week of First Observation	95	104	86
Life Table Tests (d)	P=0.362N	P=0.254N	P=0.462N
Incidental Tumor Tests (d)	P=0.336N	P=0.292N	P=0.424N
Cochran-Armitage Trend Test (d)	P=0.398N		
Fisher Exact Test (d)		P=0.309N	P=0.500N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	5/50 (10%)	(f) 6/17 (35%)	6/50 (12%)
Adjusted Rates (b)	16.8%		17.8%
Terminal Rates (c)	4/27 (15%)		5/31 (16%)
Week of First Observation	86		67
Life Table Test (d)			P=0.584
Incidental Tumor Test (d)			P=0.602
Fisher Exact Test (d)			P=0.500

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. N indicates a negative trend or lower incidence in a dosed group.
(e) Only 19 livers, 19 spleens, and 11 lymph nodes were examined.
(f) All animals were examined grossly at the site and the lesions found were evaluated microscopically. The incidence listed represents the number of animals with lesions diagnosed as tumors divided by the number of animals with gross lesions.
(g) Includes one malignant pheochromocytoma
(h) Includes two C-cell carcinomas

TABLE B4. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Fibroma (b)	Sarcoma, Fibrosarcoma, or Neurofibrosarcoma (b)	Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma (b)
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	0/49	2/49	2/49
Pooled control group (c)	2/88	0/88	2/88
C.I. Disperse Yellow 3	0/50	1/50	1/50
D & C Red No. 9	1/50	1/50	2/50
C.I. Solvent Yellow 14	0/50	1/50	1/50
L-Ascorbic acid	1/50	0/50	1/50
TOTAL	4/337 (1.2%)	5/337 (1.5%)	9/337 (2.7%)
SD (d)	1.15%	1.53%	1.02%
Range (e)			
High	2/88	2/49	2/49
Low	0/50	0/88	1/50
Overall Historical Incidence			
TOTAL	23/2,021 (1.1%)	27/2,021 (1.3%)	50/2,021 (2.5%)
SD (d)	1.52%	1.60%	2.31%
Range (e)			
High	3/49	3/50	(f) 5/49
Low	0/50	0/88	0/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No neurofibromas, myxomas, or myxosarcomas have been observed.
(c) Common control group for C.I. Acid Orange 10, FD & C Yellow No. 6, and C.I. Acid Red 14
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) Second highest incidence: 3/50

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	46	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Ulcer, NOS	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
Inflammation chronic necrotizing	1 (2%)		
RESPIRATORY SYSTEM			
*Nasal mucosa	(50)	(50)	(50)
Inflammation, acute focal	3 (6%)		5 (10%)
Inflammation, active chronic	1 (2%)		
#Lung	(50)	(12)	(50)
Congestion, acute	1 (2%)		1 (2%)
Inflammation, acute diffuse	2 (4%)		
Pneumonia, interstitial chronic	3 (6%)	2 (17%)	5 (10%)
Granuloma, NOS			2 (4%)
Necrosis, focal			1 (2%)
Hyperplasia, epithelial	1 (2%)		3 (6%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(9)	(50)
Myelofibrosis	1 (2%)		1 (2%)
Hypoplasia, hematopoietic	1 (2%)		
#Splenic follicles	(50)	(19)	(50)
Inflammation, granulomatous focal	1 (2%)	1 (5%)	2 (4%)
Depletion, lymphoid	6 (12%)		1 (2%)
#Splenic red pulp	(50)	(19)	(50)
Fibrosis	2 (4%)		
Hemosiderosis	1 (2%)		2 (4%)
Hematopoiesis	1 (2%)	1 (5%)	4 (8%)
#Mandibular lymph node	(49)	(11)	(50)
Hemorrhage	4 (8%)	-	2 (4%)
Inflammation, granulomatous focal	4 (8%)		1 (2%)
Plasmacytosis	2 (4%)		
#Mediastinal lymph node	(49)	(11)	(50)
Hemorrhage	8 (16%)		4 (8%)
Inflammation, granulomatous focal	4 (8%)		1 (2%)
#Mesenteric lymph node	(49)	(11)	(50)
Inflammation, granulomatous focal	1 (2%)		1 (2%)
#Renal lymph node	(49)	(11)	(50)
Inflammation, granulomatous focal	1 (2%)		
#Thymus	(40)	(8)	(42)
Embryonal duct cyst			1 (2%)
Hemorrhage	1 (3%)		
#Thymic cortex	(40)	(8)	(42)
Depletion, lymphoid	33 (83%)		26 (62%)
#Thymic medulla	(40)	(8)	(42)
Multiple cysts		1 (13%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#Heart/atrium	(50)	(9)	(50)
Thrombosis, NOS	1 (2%)	1 (11%)	1 (2%)
#Myocardium	(50)	(9)	(50)
Mineralization	2 (4%)		
Inflammation, acute/chronic	1 (2%)		
Degeneration, NOS	39 (78%)	5 (56%)	30 (60%)
#Cardiac valve	(50)	(9)	(50)
Inflammation, pyogranulomatous	1 (2%)		
*Aorta	(50)	(50)	(50)
Aneurysm	1 (2%)		
Inflammation, acute focal	1 (2%)		
Inflammation, chronic focal	1 (2%)		
*Sup. pancreaticoduodenal artery	(50)	(50)	(50)
Inflammation, active chronic			2 (4%)
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Hyperplasia, epithelial			1 (2%)
Hyperkeratosis			1 (2%)
*Root of tooth	(50)	(50)	(50)
Inflammation, acute focal		1 (2%)	
*Gum of maxilla	(50)	(50)	(50)
Inflammation, acute/chronic		1 (2%)	
*Periodontal tissues	(50)	(50)	(50)
Inflammation, suppurative	2 (4%)		
#Salivary gland	(50)	(10)	(49)
Inflammation, acute focal	1 (2%)		
Inflammation, chronic focal	1 (2%)		
Necrosis, diffuse	1 (2%)		
Atrophy, focal	1 (2%)		
Hyperplasia, focal	1 (2%)		
#Liver	(50)	(19)	(50)
Congestion, NOS			1 (2%)
Inflammation, granulomatous focal	29 (58%)	2 (11%)	33 (66%)
Degeneration, cystic			2 (4%)
Necrosis, focal	2 (4%)	1 (5%)	1 (2%)
Basophilic cyto change	38 (76%)	11 (58%)	39 (78%)
Focal cellular change	1 (2%)		
Clear cell change	3 (6%)	1 (5%)	5 (10%)
Angiectasis	3 (6%)		
Nodular regeneration			2 (4%)
#Liver/hepatocytes	(50)	(19)	(50)
Cytoplasmic vacuolization	14 (28%)	7 (37%)	14 (28%)
#Bile duct	(50)	(19)	(50)
Hyperplasia, focal	4 (8%)	1 (5%)	9 (18%)
#Pancreas	(49)	(9)	(49)
Dilatation/ducts			1 (2%)
#Pancreatic acinus	(49)	(9)	(49)
Focal cellular change			1 (2%)
Atrophy, focal	8 (16%)	5 (56%)	12 (24%)
#Glandular stomach	(49)	(10)	(48)
Mineralization	1 (2%)		
Ulcer, NOS	4 (8%)		1 (2%)
Inflammation, acute focal	5 (10%)		1 (2%)
Necrosis, focal	1 (2%)	1 (10%)	3 (6%)
#Forestomach	(49)	(10)	(48)
Ulcer, NOS	2 (4%)		1 (2%)
Inflammation, acute focal	3 (6%)		2 (4%)
Inflammation, acute/chronic	2 (4%)		
Necrosis, focal	1 (2%)		1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#Duodenum	(48)	(9)	(49)
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, acute focal	1 (2%)		
#Colon	(50)	(10)	(49)
Parasitism	1 (2%)	1 (10%)	2 (4%)
#Cecum	(50)	(10)	(49)
Necrosis, focal		1 (10%)	
*Rectum	(50)	(50)	(50)
Parasitism			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(10)	(50)
Mineralization	4 (8%)		3 (6%)
Lymphocytic inflammatory infiltrate	2 (4%)		
Inflammation, acute necrotizing	4 (8%)		
Nephropathy	38 (76%)	4 (40%)	39 (78%)
#Kidney/cortex	(50)	(10)	(50)
Cyst, NOS	1 (2%)		
#Kidney/pelvis	(50)	(10)	(50)
Hyperplasia, epithelial	1 (2%)		1 (2%)
#Urinary bladder	(50)	(8)	(50)
Calculus, gross observation only			1 (2%)
Hyperplasia, epithelial	4 (8%)		2 (4%)
#Urinary bladder/mucosa	(50)	(8)	(50)
Metaplasia, squamous			1 (2%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(50)	(30)	(49)
Embryonal duct cyst			1 (2%)
Cyst, NOS	5 (10%)	1 (3%)	5 (10%)
Multiple cysts	11 (22%)	5 (17%)	14 (29%)
Hemorrhage	1 (2%)	1 (3%)	
Necrosis, focal	1 (2%)		
Hyperplasia, focal	4 (8%)	6 (20%)	6 (12%)
Angiectasis		4 (13%)	1 (2%)
#Adrenal/capsule	(50)	(11)	(48)
Ectopia	2 (4%)		
#Adrenal cortex	(50)	(11)	(48)
Congestion, NOS		1 (9%)	
Necrosis, focal	4 (8%)		2 (4%)
Metamorphosis, fatty	15 (30%)	3 (27%)	15 (31%)
Focal cellular change	6 (12%)	1 (9%)	9 (19%)
Hyperplasia, focal	12 (24%)	4 (36%)	18 (38%)
#Adrenal medulla	(50)	(11)	(48)
Lymphocytic inflammatory infiltrate	1 (2%)		
Focal cellular change	1 (2%)		
Hyperplasia, focal	4 (8%)		4 (8%)
#Thyroid	(50)	(11)	(47)
Follicular cyst, NOS			1 (2%)
Hyperplasia, C-cell	35 (70%)	3 (27%)	35 (74%)
Hyperplasia, follicular cell			1 (2%)
#Parathyroid	(42)	(7)	(41)
Hyperplasia, focal			1 (2%)

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Inflammation, active chronic	1 (2%)		
Hyperplasia, cystic	35 (70%)	8 (16%)	45 (90%)
*Clitoral gland	(50)	(50)	(50)
Dilatation/ducts	4 (8%)	1 (2%)	4 (8%)
Lymphocytic inflammatory infiltrate			1 (2%)
Abscess, NOS		1 (2%)	
Inflammation, granulomatous	2 (4%)	1 (2%)	1 (2%)
Inflammation, granulomatous focal	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, focal	6 (12%)		9 (18%)
#Uterus	(50)	(17)	(50)
Dilatation, NOS	2 (4%)	1 (6%)	3 (6%)
Hemorrhage	1 (2%)	2 (12%)	
Inflammation, acute/chronic	1 (2%)		1 (2%)
Hyperplasia, epithelial		1 (6%)	
#Cervix uteri	(50)	(17)	(50)
Diverticulum	1 (2%)		2 (4%)
Inflammation, acute/chronic	1 (2%)		1 (2%)
#Endometrial gland	(50)	(17)	(50)
Hyperplasia, cystic	10 (20%)	1 (6%)	6 (12%)
#Ovary	(50)	(13)	(50)
Follicular cyst, NOS	3 (6%)		3 (6%)
Parovarian cyst	3 (6%)	4 (31%)	2 (4%)
Inflammation, granulomatous focal		1 (8%)	
Necrosis, focal			1 (2%)
Atrophy, NOS	1 (2%)		
NERVOUS SYSTEM			
#Cerebral ventricle	(50)	(9)	(50)
Hydrocephalus, NOS	5 (10%)		3 (6%)
#Brain	(50)	(9)	(50)
Hemorrhage	2 (4%)		2 (4%)
Necrosis, focal			1 (2%)
Atrophy, pressure	8 (16%)		9 (18%)
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Microphthalmia	1 (2%)		
Hemorrhage			1 (2%)
*Eye/retina	(50)	(50)	(50)
Displacement, NOS			1 (2%)
Atrophy, focal	3 (6%)	1 (2%)	4 (8%)
*Eye/crystalline lens	(50)	(50)	(50)
Cataract	4 (8%)	1 (2%)	4 (8%)
MUSCULOSKELETAL SYSTEM			
*Cortex of bone	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		2 (4%)
*Metacarpal	(50)	(50)	(50)
Hyperostosis		1 (2%)	
*Metatarsal	(50)	(50)	(50)
Hyperostosis		1 (2%)	

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
*Peritoneum	(50)	(50)	(50)
Necrosis, fat		1 (2%)	
*Pleura	(50)	(50)	(50)
Congestion, acute		1 (2%)	
Inflammation, acute/chronic	1 (2%)	1 (2%)	
*Mesentery	(50)	(50)	(50)
Inflammation, active chronic			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Bacterial septicemia	2 (4%)		
SPECIAL MORPHOLOGY SUMMARY			
Necropsy perf/no histo performed		4	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE

IN THE TWO-YEAR FEED STUDY OF ROTENONE

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TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	48	50	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(50)
Sarcoma, NOS	1 (2%)	1 (2%)	1 (2%)
Fibroma		1 (2%)	
Fibrosarcoma	6 (12%)	2 (4%)	
Neurofibrosarcoma	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#Lung	(47)	(50)	(50)
Hepatocellular carcinoma, metastatic	5 (11%)	1 (2%)	
Alveolar/bronchiolar adenoma	5 (11%)	12 (24%)	6 (12%)
Alveolar/bronchiolar carcinoma	1 (2%)		2 (4%)
Sarcoma, NOS	1 (2%)		
Fibrosarcoma, metastatic	1 (2%)		
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(50)	(50)
Malignant lymphoma, undiffer type	1 (2%)		
Malignant lymphoma, lymphocytic type		3 (6%)	
Malignant lymphoma, histiocytic type			3 (6%)
Malignant lymphoma, mixed type		2 (4%)	
#Spleen	(45)	(13)	(48)
Sarcoma, NOS			1 (2%)
Malignant lymphoma, lymphocytic type			1 (2%)
#Abdominal lymph node	(42)	(19)	(49)
Malignant lymphoma, undiffer type	1 (2%)		
#Mesenteric lymph node	(42)	(19)	(49)
Malignant lymphoma, mixed type		1 (5%)	
#Peyer's patch	(44)	(6)	(50)
Malignant lymphoma, mixed type			1 (2%)
#Kidney	(47)	(10)	(50)
Malignant lymphoma, lymphocytic type		1 (10%)	
CIRCULATORY SYSTEM			
*Subcutaneous tissue	(49)	(50)	(50)
Hemangiosarcoma	1 (2%)		
#Spleen	(45)	(13)	(48)
Hemangiosarcoma			1 (2%)
DIGESTIVE SYSTEM			
#Liver	(47)	(49)	(50)
Hepatocellular adenoma	7 (15%)	9 (18%)	1 (2%)
Hepatocellular carcinoma	6 (13%)	3 (6%)	
Neurofibrosarcoma, metastatic	1 (2%)		
#Forestomach	(45)	(5)	(49)
Squamous cell papilloma	1 (2%)		
#Jejunum	(44)	(6)	(50)
Adenocarcinoma, NOS	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Adrenal/capsule	(47)	(9)	(50)
Adenoma, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
#Testis	(46)	(10)	(50)
Interstitial cell tumor			1 (2%)
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(50)	(50)
Papillary cystadenoma, NOS	3 (6%)	1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Abdominal cavity	(49)	(50)	(50)
Sarcoma, NOS			1 (2%)
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	15	13	2
Moribund sacrifice	6	1	1
Terminal sacrifice	29	36	47
TUMOR SUMMARY			
Total animals with primary tumors**	25	26	18
Total primary tumors	37	36	22
Total animals with benign tumors	13	20	10
Total benign tumors	17	23	10
Total animals with malignant tumors	19	12	10
Total malignant tumors	20	13	12
Total animals with secondary tumors##	7	1	
Total secondary tumors	7	1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE: UNTREATED CONTROL

ANIMAL NUMBER	031	048	011	001	087	013	089	021	014	056	038	037	021	015	023	016	017	046	026	043	033	002	058	084	005
WEEKS ON STUDY	010	015	018	019	019	028	028	033	034	035	046	072	076	083	088	088	089	094	098	101	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	A	+	+	N	+	+	N	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									
Fibrosarcoma														X				X	X	X					
Hemangiosarcoma																									
Neurofibrosarcoma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic													X		X										
Alveolar/bronchiolar adenoma																						X			
Alveolar/bronchiolar carcinoma																									
Sarcoma, NOS													X												
Fibrosarcoma, metastatic														X											
Trachea	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	A	+	–	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	A	+	–	A	A	+	A	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, undiffer type																	X								
Thymus	A	+	+	A	+	+	A	+	+	+	+	–	+	–	+	–	+	+	–	–	–	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	A	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Liver	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																	X		X					X	
Hepatocellular carcinoma													X		X								X	X	
Neurofibrosarcoma, metastatic												X													
Bile duct	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	A	+	N	N	N	+	N	+	+	+	N	N	N	N	+	+	N	+	+	N	N	+	+	+	+
Pancreas	A	+	–	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
Esophagus	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	A	+	–	A	+	+	A	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																						X			
Small intestine	A	+	–	A	+	+	A	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Large intestine	A	+	–	A	A	+	A	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	–	–	A	A	+	A	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	A	+	+	A	A	+	A	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+
Adrenal	A	+	–	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Thyroid	A	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	A	–	–	A	A	+	A	+	+	+	–	–	+	+	+	+	–	–	–	–	+	–	+	+	–
REPRODUCTIVE SYSTEM																									
Mammary gland	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	A	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	A	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	A	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Papillary cystadenoma, NOS																								X	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, undiffer type																		X							

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed
: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0110	0112	0118	0119	0120	0121	0123	0124	0127	0128	0129	0130	0134	0135	0136	0138	0139	0140	0141	0142	0144	0145	0147	0149	0150	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	105	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Sarcoma, NOS																	X		X							1
Fibrosarcoma																	X				X					6
Hemangiosarcoma																								X		1
Neurofibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Hepatocellular carcinoma, metastatic			X									X											X			5
Alveolar/bronchiolar adenoma	X																	X	X							5
Alveolar/bronchiolar carcinoma																									X	1
Sarcoma, NOS																										1
Fibrosarcoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lymph nodes	+	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Malig. lymphoma, undiffer type																										1
Thymus	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	–	+	–	–	+	+	–	+	+	35
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	47
Hepatocellular adenoma							X		X														X		X	7
Hepatocellular carcinoma			X									X														6
Neurofibrosarcoma, metastatic																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder & common bile duct	N	N	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Squamous cell papilloma																										1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Adenocarcinoma, NOS									X																	1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma, NOS								X																		1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Parathyroid	–	+	+	–	+	+	–	–	+	+	+	–	+	+	–	+	–	+	+	+	+	+	–	+	+	28
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	[illegible]	N	N	N	N	N	N	N	N	N	N	*49
Papillary cystadenoma, NOS			X																				X			3
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Malignant lymphoma, undiffer type																										1

* Animals necropsied

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE: LOW DOSE

ANIMAL NUMBER	0 0 2 4	0 0 3 5	0 0 4 6	0 0 4 1	0 0 1 2	0 0 1 3	0 0 1 7	0 0 2 1	0 0 2 2	0 0 2 3	0 0 2 9	0 0 3 0	0 0 3 1	0 0 3 2	0 0 3 3	0 0 3 4	0 0 3 5	0 0 3 6	0 0 3 7	0 0 3 8	0 0 3 9	0 0 4 0	0 0 4 1	0 0 4 2	0 0 4 3	0 1 0 4
WEEKS ON STUDY	0 1 4	0 2 0	0 2 3	0 3 2	0 3 6	0 3 2	0 3 8	0 4 7	0 5 0	0 5 0	0 5 0	0 5 0	0 5 7	0 5 8	0 5 8	0 6 1	0 6 4	0 6 4	0 6 4	0 6 4	0 6 4	0 6 4	0 6 4	0 6 4	0 6 4	0 6 4	1 0 4
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	+	N	N	N	+	+	N	N	N	N	N
Sarcoma, NOS																					X					
Fibroma																										
Fibrosarcoma									X											X						
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic																										
Alveolar/bronchiolar adenoma							X						X				X									
Trachea	–	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	+	+	–	+	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Lymph nodes	+	–	–	+	–	–	–	+	+	+	–	+	+	+	–	–	–	–	+	–	–	+	+	–	–	+
Malignant lymphoma, mixed type																										X
Thymus	+	+	–	–	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DIGESTIVE SYSTEM																										
Salivary gland	+	–	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma									X		X															X
Hepatocellular carcinoma								X									X			X						
Bile duct	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	+	N	N	N	N	N	+	N	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Esophagus	+	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	–	+	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Small intestine	–	+	–	–	–	–	+	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Large intestine	+	+	–	+	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																										
Kidney	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Malignant lymphoma, lymphocytic type																										
Urinary bladder	+	+	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																										
Pituitary	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Adrenal	+	+	–	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thyroid	+	+	+	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Parathyroid	–	–	+	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Prostate	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NERVOUS SYSTEM																										
Brain	+	–	+	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Papillary cystadenoma, NOS																										
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	X	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type													X	X												
Malignant lymphoma, mixed type												X														

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	018	019	020	023	024	025	026	027	028	029	030	031	033	034	036	038	040	042	043	044	045	047	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	*50
Sarcoma, NOS																										1
Fibroma									X																	1
Fibrosarcoma																										2
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma				X		X							X	X		X	X				X			X	X	12
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Spleen	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	13
Lymph nodes	–	–	–	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	+	–	+	+	+	–	19
Malignant lymphoma, mixed type																										1
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma		X							X				X								X		X		X	9
Hepatocellular carcinoma																										3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	8
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
Small intestine	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	10
Malignant lymphoma, lymphocytic type																								X		1
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
ENDOCRINE SYSTEM																										
Pituitary	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
Prostate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Papillary cystadenoma, NOS			X																							1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, lymphocytic type																							X			3
Malignant lymphoma, mixed type		X																								2

* Animals necropsied

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE: HIGH DOSE

ANIMAL NUMBER	001	002	003	006	009	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023
WEEKS ON STUDY	041	056	058	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																								X	
Neurofibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X						X					
Alveolar/bronchiolar carcinoma				X								X													
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Sarcoma, NOS								X																	
Hemangiosarcoma																									
Malignant lymphoma, lymphocytic type																									
Lymph nodes	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	–	+	–	–	–	+	+	+	+	+	–	+	+	+	–	+	+	–	+	+	+	–	–	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, mixed type																			X						
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	–	–	+	+	–	+	+	+	–	+	–	+	+	+	–	–	+	+	+	+	+	+	+	+	–
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Papillary cystadenoma, NOS								X																	
BODY CAVITIES																									
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS			X																						
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, histiocytic type		X						X				X													

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	0 2 4	0 2 8	0 3 1	0 3 7	0 3 9	0 4 0	0 4 1	0 4 2	0 4 4	0 4 5	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	0 5 1	0 5 2	0 5 3	0 5 4	0 5 5	0 5 6	0 5 7	0 5 8	0 5 9	0 6 0	TOTAL:
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	TISSUES / TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS															X											1
Neurofibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma					X						X				X				X							6
Alveolar/bronchiolar carcinoma																										2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Sarcoma, NOS																										1
Hemangiosarcoma																									X	1
Malignant lymphoma, lymphocytic type	X																									1
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	37
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma								X																		1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Malignant lymphoma, mixed type																										1
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	–	+	+	+	–	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	43
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	49
Parathyroid	+	+	–	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	–	+	–	–	+	+	34
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor									X																	1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Papillary cystadenoma, NOS		X																								2
BODY CAVITIES																										
Peritoneum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, histiocytic type																										3

* Animals necropsied

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control	600 ppm	1,200 ppm
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	6/49 (12%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	18.0%	5.1%	0.0%
Terminal Rates (c)	2/29 (7%)	1/36 (3%)	0/47 (0%)
Week of First Observation	83	88	
Life Table Tests (d)	P=0.002N	P=0.087N	P=0.004N
Incidental Tumor Tests (d)	P=0.008N	P=0.086N	P=0.017N
Cochran-Armitage Trend Test (d)	P=0.007N		
Fisher Exact Test (d)		P=0.128N	P=0.012N
Subcutaneous Tissue: Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	8/49 (16%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	23.1%	7.8%	4.3%
Terminal Rates (c)	3/29 (10%)	2/36 (6%)	2/47 (4%)
Week of First Observation	72	88	104
Life Table Tests (d)	P=0.005N	P=0.059N	P=0.010N
Incidental Tumor Tests (d)	P=0.028N	P=0.054N	P=0.070N
Cochran-Armitage Trend Test (d)	P=0.023N		
Fisher Exact Test (d)		P=0.094N	P=0.043N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	6/49 (12%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	18.0%	7.8%	0.0%
Terminal Rates (c)	2/29 (7%)	2/36 (6%)	0/47 (0%)
Week of First Observation	83	88	
Life Table Tests (d)	P=0.002N	P=0.162N	P=0.004N
Incidental Tumor Tests (d)	P=0.011N	P=0.171N	P=0.017N
Cochran-Armitage Trend Test (d)	P=0.009N		
Fisher Exact Test (d)		P=0.233N	P=0.012N
Subcutaneous Tissue: Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	8/49 (16%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	23.1%	10.5%	4.3%
Terminal Rates (c)	3/29 (10%)	3/36 (8%)	2/47 (4%)
Week of First Observation	72	88	104
Life Table Tests (d)	P=0.006N	P=0.107N	P=0.010N
Incidental Tumor Tests (d)	P=0.030N	P=0.104N	P=0.070N
Cochran-Armitage Trend Test (d)	P=0.027N		
Fisher Exact Test (d)		P=0.168N	P=0.043N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	5/47 (11%)	12/50 (24%)	6/50 (12%)
Adjusted Rates (b)	17.2%	31.2%	12.8%
Terminal Rates (c)	5/29 (17%)	10/36 (28%)	6/47 (13%)
Week of First Observation	104	78	104
Life Table Test (d)	P=0.275N	P=0.129	P=0.420N
Incidental Tumor Test (d)	P=0.347N	P=0.136	P=0.420N
Fisher Exact Test (d)	P=0.497	P=0.071	P=0.544
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	6/47 (13%)	12/50 (24%)	8/50 (16%)
Adjusted Rates (b)	20.7%	31.2	17.0%
Terminal Rates (c)	6/29 (21%)	10/36 (28%)	8/47 (17%)
Week of First Observation	104	78	104
Life Table Test (d)	P=0.333N	P=0.207	P=0.462N
Incidental Tumor Test (d)	P=0.408N	P=0.217	P=0.462N
Fisher Exact Test (d)	P=0.398	P=0.122	P=0.436

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	600 ppm	1,200 ppm
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	0/49 (0%)	(e) 0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	0.0%		6.2%
Terminal Rates (c)	0/29 (0%)		2/47 (4%)
Week of First Observation			96
Life Table Test (d)			P=0.219
Incidental Tumor Test (d)			P=0.152
Fisher Exact Test (d)			P=0.125
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	2/50 (4%)	(e) 7/50 (14%)	5/50 (10%)
Adjusted Rates (b)	5.9%		10.4%
Terminal Rates (c)	0/29 (0%)		4/47 (9%)
Week of First Observation	89		96
Life Table Test (d)			P=0.427
Incidental Tumor Test (d)			P=0.064
Fisher Exact Test (d)			P=0.218
Liver: Hepatocellular Adenoma			
Overall Rates (a)	7/47 (15%)	9/49 (18%)	1/50 (2%)
Adjusted Rates (b)	22.2%	23.3%	2.1%
Terminal Rates (c)	5/29 (17%)	7/36 (19%)	1/47 (2%)
Week of First Observation	89	88	104
Life Table Tests (d)	P=0.005N	P=0.575	P=0.006N
Incidental Tumor Tests (d)	P=0.019N	P=0.532	P=0.018N
Cochran-Armitage Trend Test (d)	P=0.032N		
Fisher Exact Test (d)		P=0.428	P=0.024N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	6/47 (13%)	3/49 (6%)	0/50 (0%)
Adjusted Rates (b)	18.5%	7.8%	0.0%
Terminal Rates (c)	4/29 (14%)	2/36 (6%)	0/47 (0%)
Week of First Observation	76	87	
Life Table Tests (d)	P=0.002N	P=0.157N	P=0.004N
Incidental Tumor Tests (d)	P=0.009N	P=0.184N	P=0.019N
Cochran-Armitage Trend Test (d)	P=0.008N		
Fisher Exact Test (d)		P=0.223N	P=0.011N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	12/47 (26%)	12/49 (24%)	1/50 (2%)
Adjusted Rates (b)	35.6%	30.3%	2.1%
Terminal Rates (c)	8/29 (28%)	9/36 (25%)	1/47 (2%)
Week of First Observation	76	87	104
Life Table Tests (d)	P<0.001N	P=0.365N	P<0.001N
Incidental Tumor Tests (d)	P=0.001N	P=0.429N	P=0.001N
Cochran-Armitage Trend Test (d)	P=0.001N		
Fisher Exact Test (d)		P=0.546N	P<0.001N
Harderian Gland: Papillary Cystadenoma			
Overall Rates (a)	3/49 (6%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	10.3%	2.8%	4.3%
Terminal Rates (c)	3/29 (10%)	1/36 (3%)	2/47 (4%)
Week of First Observation	104	104	104
Life Table Tests (d)	P=0.224N	P=0.231N	P=0.288N
Incidental Tumor Tests (d)	P=0.224N	P=0.231N	P=0.288N
Cochran-Armitage Trend Test (d)	P=0.391N		
Fisher Exact Test (d)		P=0.301N	P=0.490N

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. N indicates a negative trend or lower incidence in a dosed group.
(e) Only 13 spleens and 19 lymph nodes were examined.

TABLE C4a. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Fibroma or Neurofibroma	Sarcoma, Fibrosarcoma, or Neurofibrosarcoma	Fibroma, Neurofibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	1/50	1/50	2/50
C.I. Acid Orange 10	0/50	6/50	6/50
FD & C Yellow No. 6	0/50	4/50	4/50
C.I. Acid Red 14	0/49	4/49	4/49
C.I. Disperse Yellow 3	0/50	0/50	0/50
D & C Red No. 9	0/50	2/50	2/50
C.I. Solvent Yellow 14	0/49	0/49	0/49
L-Ascorbic acid	0/50	1/50	1/50
TOTAL	1/398 (0.3%)	18/398 (4.5%)	19/398 (4.8%)
SD (b)	0.71%	4.39%	4.29%
Range (c)			
High	1/50	6/50	6/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	36/2,091 (1.7%)	125/2,091 (6.0%)	156/2,091 (7.5%)
SD (b)	2.78%	6.46%	7.68%
Range (c)			
High	6/50	15/50	19/50
Low	0/50	0/50	0/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE C4b. HISTORICAL INCIDENCE OF HEPATOCELLULAR TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Battelle Columbus Laboratories			
Chlorobenzene	7/50	14/50	19/50
C.I. Acid Orange 10	1/50	14/50	15/50
FD & C Yellow No. 6	1/50	13/50	13/50
C.I. Acid Red 14	6/48	10/48	15/48
C.I. Disperse Yellow 3	7/50	14/50	20/50
D & C Red No. 9	4/50	4/50	8/50
C.I. Solvent Yellow 14	5/49	10/49	15/49
L-Ascorbic acid	6/50	10/50	16/50
TOTAL	37/397 (9.3%)	89/397 (22.4%)	121/397 (30.5%)
SD (b)	4.94%	6.83%	7.37%
Range (c)			
High	7/50	14/50	20/50
Low	1/50	4/50	8/50
Overall Historical Incidence			
TOTAL	228/2,084 (10.9%)	424/2,084 (20.3%)	627/2,084 (30.1%)
SD (b)	7.29%	6.85%	7.78%
Range (c)			
High	(d) 22/50	16/50	(e) 29/50
Low	0/49	4/50	8/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Second highest: 11/50
(e) Second highest: 20/50

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	48	50	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(50)	(50)
Ulcer, NOS	1 (2%)	1 (2%)	
Inflammation, acute/chronic	1 (2%)		
Inflammation, chronic focal	1 (2%)		
Hyperplasia, NOS			1 (2%)
*Subcutaneous tissue	(49)	(50)	(50)
Inflammation, acute/chronic	3 (6%)	1 (2%)	1 (2%)
Fibrosis	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
#Lung/bronchiole	(47)	(50)	(50)
Foreign body, NOS			1 (2%)
#Lung	(47)	(50)	(50)
Hemorrhage	2 (4%)		
Lymphocytic inflammatory infiltrate	1 (2%)		
Inflammation, interstitial	2 (4%)	6 (12%)	1 (2%)
Hyperplasia, epithelial	4 (9%)	3 (6%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(48)	(10)	(50)
Hyperplasia, granulocytic	8 (17%)	3 (30%)	
#Splenic follicles	(45)	(13)	(48)
Necrosis, focal	1 (2%)	2 (15%)	
Depletion, lymphoid	2 (4%)	2 (15%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)
#Splenic red pulp	(45)	(13)	(48)
Hematopoiesis	13 (29%)	7 (54%)	3 (6%)
#Mandibular lymph node	(42)	(19)	(49)
Hemorrhage			1 (2%)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, plasma cell		1 (5%)	
#Mediastinal lymph node	(42)	(19)	(49)
Inflammation, acute focal	1 (2%)		
#Abdominal lymph node	(42)	(19)	(49)
Hyperplasia, lymphoid			2 (4%)
#Pancreatic lymph node	(42)	(19)	(49)
Inflammation, chronic focal	1 (2%)		
#Lumbar lymph node	(42)	(19)	(49)
Hyperplasia, lymphoid			1 (2%)
#Mesenteric lymph node	(42)	(19)	(49)
Inflammation, chronic focal	2 (5%)		
Hyperplasia, lymphoid		4 (21%)	1 (2%)
Hematopoiesis	14 (33%)	8 (42%)	12 (24%)
#Renal lymph node	(42)	(19)	(49)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, lymphoid	1 (2%)		
#Brachial lymph node	(42)	(19)	(49)
Hyperplasia, lymphoid	1 (2%)		
#Inguinal lymph node	(42)	(19)	(49)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, lymphoid	4 (10%)		

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#Liver	(47)	(49)	(50)
Hematopoiesis	6 (13%)	2 (4%)	
#Peyer's patch	(44)	(6)	(50)
Hyperplasia, lymphoid		1 (17%)	2 (4%)
#Thymus	(35)	(6)	(37)
Ultimobranchial cyst	1 (3%)		
Depletion, lymphoid	5 (14%)		
#Thymic lymphocytes	(35)	(6)	(37)
Necrosis, focal	1 (3%)	3 (50%)	
CIRCULATORY SYSTEM			
#Heart/atrium	(47)	(10)	(50)
Thrombosis, NOS			1 (2%)
#Left ventricle	(47)	(10)	(50)
Embolism, NOS	1 (2%)		
#Myocardium	(47)	(10)	(50)
Inflammation, acute focal	1 (2%)	1 (10%)	
DIGESTIVE SYSTEM			
#Liver	(47)	(49)	(50)
Inflammation, acute focal	1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic focal	3 (6%)		1 (2%)
Necrosis, focal	3 (6%)	1 (2%)	
Basophilic cyto change		2 (4%)	
Focal cellular change			1 (2%)
#Liver/hepatocytes	(47)	(49)	(50)
Nuclear alteration		1 (2%)	
#Pancreas	(45)	(8)	(48)
Dilatation/ducts		1 (13%)	
Inflammation, acute focal	1 (2%)		
#Pancreatic acinus	(45)	(8)	(48)
Eosinophilic cyto change	1 (2%)		
Atrophy, focal	2 (4%)		
#Glandular stomach	(45)	(5)	(49)
Diverticulum	1 (2%)		
Inflammation, acute focal	2 (4%)		1 (2%)
Inflammation, chronic focal	1 (2%)		
#Forestomach	(45)	(5)	(49)
Hyperkeratosis	1 (2%)		
Acanthosis	1 (2%)		
#Colon	(43)	(6)	(50)
Parasitism	2 (5%)		
*Transition zone of anal mucous membrane	(49)	(50)	(50)
Inflammation, active chronic			2 (4%)
Hyperplasia, epithelial			2 (4%)
URINARY SYSTEM			
#Kidney	(47)	(10)	(50)
Inflammation, acute focal		2 (20%)	
Glomerulonephritis, chronic	1 (2%)		1 (2%)
Inflammation, chronic focal	6 (13%)	2 (20%)	3 (6%)
Nephropathy	2 (4%)	1 (10%)	
#Kidney/cortex	(47)	(10)	(50)
Cyst, NOS	1 (2%)	1 (10%)	
Multiple cysts	1 (2%)		

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM (Continued)			
#Kidney/tubule	(47)	(10)	(50)
Mineralization		2 (20%)	
Dilatation, NOS	1 (2%)		
Cytoplasmic vacuolization		1 (10%)	
Regeneration, NOS	22 (47%)		33 (66%)
#Kidney/pelvis	(47)	(10)	(50)
Inflammation, suppurative	2 (4%)	2 (20%)	
#Urinary bladder	(43)	(7)	(50)
Distention	2 (5%)		
Inflammation, acute focal			1 (2%)
Inflammation, acute/chronic	1 (2%)	2 (29%)	
Inflammation, chronic focal		1 (14%)	
Hyperplasia, epithelial		1 (14%)	
*Urethra	(49)	(50)	(50)
Obstruction, NOS		3 (6%)	
Inflammation, acute necrotizing		1 (2%)	
Inflammation, acute/chronic		1 (2%)	
ENDOCRINE SYSTEM			
#Adrenal/capsule	(47)	(9)	(50)
Cytoplasmic vacuolization			1 (2%)
Hyperplasia, focal	38 (81%)		48 (96%)
#Adrenal cortex	(47)	(9)	(50)
Ectopia	1 (2%)		
Focal cellular change	1 (2%)		5 (10%)
Hyperplasia, focal	1 (2%)		
#Adrenal medulla	(47)	(9)	(50)
Hyperplasia, focal	1 (2%)		
#Periadrenal tissue	(47)	(9)	(50)
Inflammation, acute/chronic	1 (2%)		
#Thyroid	(46)	(9)	(49)
Follicular cyst, NOS	2 (4%)		2 (4%)
Inflammation, acute focal			1 (2%)
Hyperplasia, follicular cell			1 (2%)
#Pancreatic islets	(45)	(8)	(48)
Hyperplasia, focal			1 (2%)
REPRODUCTIVE SYSTEM			
*Prepuce	(49)	(50)	(50)
Ulcer, NOS			2 (4%)
Inflammation, acute necrotizing	1 (2%)	2 (4%)	
Inflammation, acute/chronic		1 (2%)	1 (2%)
*Preputial gland	(49)	(50)	(50)
Dilatation/ducts		1 (2%)	
Cyst, NOS		1 (2%)	
Inflammation, suppurative	6 (12%)	9 (18%)	
Inflammation, acute/chronic	1 (2%)	1 (2%)	3 (6%)
Inflammation, chronic focal	1 (2%)		
#Prostate	(46)	(10)	(50)
Inflammation, acute focal	10 (22%)	2 (20%)	
Inflammation, acute/chronic		6 (60%)	
Inflammation, chronic diffuse	1 (2%)		
*Seminal vesicle	(49)	(50)	(50)
Retention fluid	4 (8%)	1 (2%)	
Inflammation, acute suppurative	1 (2%)		
Inflammation, acute/chronic		1 (2%)	
Inflammation, chronic focal	4 (8%)	1 (2%)	

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
#Testis	(46)	(10)	(50)
Inflammation, acute focal	1 (2%)		
Hyperplasia, interstitial cell	2 (4%)		4 (8%)
*Epididymis	(49)	(50)	(50)
Inflammation, acute suppurative	1 (2%)		
Inflammation, granulomatous focal	2 (4%)		
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Eye/crystalline lens	(49)	(50)	(50)
Cataract			1 (2%)
*Harderian gland	(49)	(50)	(50)
Hyperplasia, epithelial			1 (2%)
MUSCULOSKELETAL SYSTEM			
*Maxilla	(49)	(50)	(50)
Inflammation, acute suppurative	1 (2%)		
*Knee joint	(49)	(50)	(50)
Hyperostosis		1 (2%)	1 (2%)
Metaplasia, osseous		1 (2%)	
*Tarsal joint	(49)	(50)	(50)
Hyperostosis	8 (16%)	16 (32%)	10 (20%)
Metaplasia, osseous	8 (16%)	15 (30%)	9 (18%)
BODY CAVITIES			
*Peritoneum	(49)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
No lesion reported		2	
Auto/necropsy/histo perf	2		
Auto/necropsy/no histo	1		
Autolysis/no necropsy	1		

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE

IN THE TWO-YEAR FEED STUDY OF ROTENONE

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TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	49	50
INTEGUMENTARY SYSTEM			
*Subcutaneous tissue	(49)	(49)	(50)
Fibrosarcoma	3 (6%)	1 (2%)	1 (2%)
Liposarcoma		1 (2%)	
RESPIRATORY SYSTEM			
#Lung	(48)	(10)	(50)
Hepatocellular carcinoma, metastatic		1 (10%)	
Alveolar/bronchiolar adenoma	3 (6%)	2 (20%)	4 (8%)
Alveolar/bronchiolar carcinoma	1 (2%)		1 (2%)
Fibrosarcoma, metastatic	1 (2%)		
Liposarcoma, metastatic		1 (10%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(49)	(49)	(50)
Malignant lymphoma, undiffer type			2 (4%)
Malignant lymphoma, lymphocytic type	6 (12%)	2 (4%)	3 (6%)
Malignant lymphoma, histiocytic type	1 (2%)	2 (4%)	
Malignant lymphoma, mixed type	1 (2%)		5 (10%)
#Spleen	(48)	(12)	(49)
Malignant lymphoma, undiffer type	1 (2%)		
#Pancreatic lymph node	(48)	(7)	(47)
Fibrosarcoma, metastatic	1 (2%)		
#Thymus	(38)	(4)	(45)
Malignant lymphoma, lymphocytic type		1 (25%)	
CIRCULATORY SYSTEM			
#Ovary	(48)	(17)	(50)
Hemangioma		1 (6%)	
DIGESTIVE SYSTEM			
#Liver	(49)	(10)	(49)
Hepatocellular adenoma	3 (6%)	1 (10%)	2 (4%)
Hepatocellular carcinoma	1 (2%)	2 (20%)	2 (4%)
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Pituitary intermedia	(43)	(9)	(44)
Adenoma, NOS	1 (2%)		
#Anterior pituitary	(43)	(9)	(44)
Adenoma, NOS	3 (7%)	1 (11%)	
#Adrenal/capsule	(49)	(6)	(50)
Adenoma, NOS	1 (2%)		
#Adrenal medulla	(49)	(6)	(50)
Pheochromocytoma	1 (2%)		
#Thyroid	(48)	(6)	(49)
Follicular cell adenoma	2 (4%)		

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(49)	(50)
Adenocarcinoma, NOS	3 (6%)	2 (4%)	
#Uterus	(48)	(43)	(49)
Endometrial stromal polyp	3 (6%)	1 (2%)	3 (6%)
#Cervix uteri	(48)	(43)	(49)
Myxoma	1 (2%)		
#Ovary	(48)	(17)	(50)
Adenoma, NOS	1 (2%)	1 (6%)	
Granulosa cell tumor			1 (2%)
Sertoli cell tumor			1 (2%)
Mixed tumor, benign	1 (2%)		
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(49)	(50)
Adenoma, NOS	1 (2%)		1 (2%)
Adenocarcinoma, NOS			1 (2%)
Papillary cystadenoma, NOS	2 (4%)		2 (4%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(49)	(50)
Fibrosarcoma, metastatic		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	12	7	5
Moribund sacrifice		2	
Terminal sacrifice	37	40	45
Animal missing	1	1	

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
Total animals with primary tumors**	26	16	22
Total primary tumors	40	18	29
Total animals with benign tumors	20	7	11
Total benign tumors	23	7	13
Total animals with malignant tumors	14	11	14
Total malignant tumors	17	11	15
Total animals with secondary tumors##	1	3	
Total secondary tumors	2	3	
Total animals with tumors uncertain--benign or malignant			1
Total uncertain tumors			1

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE: UNTREATED CONTROL

ANIMAL NUMBER	043	042	048	050	015	043	030	018	046	017	035	010	049	001	002	003	004	007	008	009	011	012	013	014	018
WEEKS ON STUDY	043	051	089	099	073	079	082	088	096	098	099	011	011	044	044	044	044	044	044	044	044	044	044	044	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	N	N	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma												X	X												
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	M	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma					X					X															
Alveolar/bronchiolar carcinoma																									
Fibrosarcoma, metastatic													X												
Trachea	+	–	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	–	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	–	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, undiffer type																									
Lymph nodes	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic													X												
Thymus	–	–	+	M	+	+	–	+	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																	X								
Hepatocellular carcinoma																									
Bile duct	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	+	M	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	–	+	M	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	M	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	–	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	–	–	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	–	–	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	–	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	–	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Adenoma, NOS																X		X							
Adrenal	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Pheochromocytoma																									
Thyroid	+	–	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																									
Parathyroid	–	–	–	M	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	M	N	N	N	+	+	+	+	N	+	+	+	N	+	+	+	+	N	+	+	N	+
Adenocarcinoma, NOS										X															
Uterus	+	–	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Myxoma																									
Endometrial stromal polyp																									
Ovary	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Mixed tumor, benign																									
NERVOUS SYSTEM																									
Brain	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									X
Papillary cystadenoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, lymphocytic type					X											X									
Malignant lymphoma, histiocytic type											X														
Malignant lymphoma, mixed type																									

+: Tissue examined microscopically
–: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed

.: No tissue information submitted
C: Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B: No necropsy performed

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 1 9	0 2 6	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 4	0 4 7	0 4 8	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	
INTEGUMENTARY SYSTEM																	
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Fibrosarcoma								X									3
RESPIRATORY SYSTEM																	
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Alveolar/bronchiolar adenoma											X						3
Alveolar/bronchiolar carcinoma													X				1
Fibrosarcoma, metastatic																	1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Malignant lymphoma, undiffer type															X		1
Lymph nodes	+	+	+	+	–	+	+	–	+	+	+	+	+	+	+	+	48
Fibrosarcoma, metastatic																	1
Thymus	+	–	+	–	–	+	+	–	+	+	+	+	+	+	+	+	38
CIRCULATORY SYSTEM																	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																	
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma		X													X		3
Hepatocellular carcinoma													X				1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*49
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																	
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																	
Pituitary	+	–	+	+	+	–	+	+	+	+	–	+	+	+	+	+	43
Adenoma, NOS							X					X					4
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS						X											1
Pheochromocytoma														X			1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma															X	X	2
Parathyroid	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	42
REPRODUCTIVE SYSTEM																	
Mammary gland	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	+	*49
Adenocarcinoma, NOS	X		X														3
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Myxoma														X			1
Endometrial stromal polyp		X								X		X					3
Ovary	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS																X	1
Mixed tumor, benign				X													1
NERVOUS SYSTEM																	
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																	
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenoma, NOS																	1
Papillary cystadenoma, NOS			X							X							2
ALL OTHER SYSTEMS																	
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Malignant lymphoma, lymphocytic type	X	X												X			6
Malignant lymphoma, histiocytic type							X										1
Malignant lymphoma, mixed type																	1

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE: LOW DOSE

ANIMAL NUMBER	028	020	047	035	029	033	041	014	001	002	003	004	005	006	007	008	009	010	011	012	013	015	016	017	018
WEEKS ON STUDY	012	052	066	069	073	078	083	089	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	M	+	+	+	N	N	+	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma		X					X																		
Liposarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	M	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Hepatocellular carcinoma, metastatic								X																	
Alveolar/bronchiolar adenoma		X																							
Liposarcoma, metastatic																									
Trachea	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HEMATOPOIETIC SYSTEM																									
Bone marrow	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen	M	+	+	+	A	+	+	+	–	–	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–
Lymph nodes	M	–	+	+	A	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thymus	M	+	–	+	A	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Malignant lymphoma, lymphocytic type																						X			
CIRCULATORY SYSTEM																									
Heart	M	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DIGESTIVE SYSTEM																									
Salivary gland	M	+	+	+	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver	M	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hepatocellular adenoma								X																	
Hepatocellular carcinoma																									
Bile duct	M	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Gallbladder & common bile duct	M	+	+	+	+	+	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pancreas	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–
Esophagus	M	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Small intestine	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Large intestine	M	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
URINARY SYSTEM																									
Kidney	M	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Urinary bladder	M	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ENDOCRINE SYSTEM																									
Pituitary	M	+	+	+	A	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Adenoma, NOS																									
Adrenal	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thyroid	M	+	+	+	A	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Parathyroid	M	+	+	–	A	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
REPRODUCTIVE SYSTEM																									
Mammary gland	M	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS																	X								
Uterus	M	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp				X																					
Ovary	M	+	+	+	A	+	+	+	–	–	–	+	–	–	–	–	+	–	–	–	+	–	+	+	–
Adenoma, NOS																									
Hemangioma								X																	
NERVOUS SYSTEM																									
Brain	M	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ALL OTHER SYSTEMS																									
Multiple organs, NOS	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma, metastatic							X																		
Malignant lymphoma, lymphocytic type												X													
Malignant lymphoma, histiocytic type						X																			

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	019	021	022	023	024	025	026	027	028	031	032	034	036	037	038	039	040	042	043	044	045	046	048	049	050	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	103	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Fibrosarcoma																										1
Liposarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	10
Hepatocellular carcinoma, metastatic																										1
Alveolar/bronchiolar adenoma																	X								X	2
Liposarcoma, metastatic																										1
Trachea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
HEMATOPOIETIC SYSTEM																										
Bone marrow	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Spleen	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	12
Lymph nodes	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Thymus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4
Malignant lymphoma, lymphocytic type																										1
CIRCULATORY SYSTEM																										
Heart	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
DIGESTIVE SYSTEM																										
Salivary gland	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Liver	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	10
Hepatocellular adenoma																							X			1
Hepatocellular carcinoma					X																					2
Bile duct	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	10
Gallbladder & common bile duct	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Pancreas	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	10
Esophagus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
Stomach	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Small intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Large intestine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
URINARY SYSTEM																										
Kidney	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	9
Urinary bladder	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
ENDOCRINE SYSTEM																										
Pituitary	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9
Adenoma, NOS		X																								1
Adrenal	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Thyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
Parathyroid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	*49
Adenocarcinoma, NOS													X													2
Uterus	+	+	+	–	–	+	+	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	+	+	+	43
Endometrial stromal polyp																										1
Ovary	–	–	+	+	–	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	+	–	–	–	–	17
Adenoma, NOS																X										1
Hemangioma																										1
NERVOUS SYSTEM																										
Brain	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*49
Fibrosarcoma, metastatic																										1
Malignant lymphoma, lymphocytic type																									X	2
Malignant lymphoma, histiocytic type				X																						2

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE: HIGH DOSE

ANIMAL NUMBER	043	032	037	020	045	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	021
WEEKS ON STUDY	060	087	097	093	098	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																						X			
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma													X		X		X								
Alveolar/bronchiolar carcinoma																X									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Thymus	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	–	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Liver	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular carcinoma												X					X								
Bile duct	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+
Pancreas	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	–	–	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thyroid	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid	–	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	–	+	–	+	+	–
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	N	N	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+	+	+
Uterus	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp													X						X					X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor																X									
Sertoli cell tumor																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Adenocarcinoma, NOS							X																		
Papillary cystadenoma, NOS																		X						X	
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, undiffer type																								X	
Malignant lymphoma, lymphocytic type				X							X														
Malignant lymphoma, mixed type																						X			

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE
(Continued)

ANIMAL NUMBER	022	023	024	025	026	027	028	029	030	031	033	034	035	036	038	039	040	041	042	044	046	047	048	049	050	TOTAL:
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X																4
Alveolar/bronchiolar carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	47
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma																					X		X			2
Hepatocellular carcinoma																										2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid	+	+	+	–	+	+	+	+	+	–	+	–	–	+	+	+	–	+	–	+	+	+	+	+	–	36
REPRODUCTIVE SYSTEM																										
Mammary gland	N	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endometrial stromal polyp																										3
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor																										1
Sertoli cell tumor					X																					1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS			X																							1
Adenocarcinoma, NOS																										1
Papillary cystadenoma, NOS																										2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	M	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, undiffer type															X											2
Malignant lymphoma, lymphocytic type																			X							3
Malignant lymphoma, mixed type									X			X		X									X			5

* Animals necropsied

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control	600 ppm	1,200 ppm
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	3/49 (6%)	1/49 (2%)	1/50 (2%)
Adjusted Rates (b)	7.7%	2.3%	2.2%
Terminal Rates (c)	1/37 (3%)	0/42 (0%)	1/45 (2%)
Week of First Observation	101	83	104
Life Table Tests (d)	P=0.166N	P=0.284N	P=0.250N
Incidental Tumor Tests (d)	P=0.335N	P=0.731	P=0.356N
Cochran-Armitage Trend Test (d)	P=0.197N		
Fisher Exact Test (d)		P=0.309N	P=0.301N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/48 (6%)	(e) 2/10 (20%)	4/50 (8%)
Adjusted Rates (b)	7.1%		8.9%
Terminal Rates (c)	1/37 (3%)		4/45 (9%)
Week of First Observation	75		104
Life Table Test (d)			P=0.590
Incidental Tumor Test (d)			P=0.485
Fisher Exact Test (d)			P=0.523
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/48 (8%)	(e) 2/10 (20%)	5/50 (10%)
Adjusted Rates (b)	9.7%		11.1%
Terminal Rates (c)	2/37 (5%)		5/45 (11%)
Week of First Observation	75		104
Life Table Test (d)			P=0.606
Incidental Tumor Test (d)			P=0.514
Fisher Exact Test (d)			P=0.526
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	6/49 (12%)	(f) 3/49 (6%)	3/50 (6%)
Adjusted Rates (b)	15.4%		6.5%
Terminal Rates (c)	5/37 (14%)		2/45 (4%)
Week of First Observation	75		99
Life Table Test (d)			P=0.168N
Incidental Tumor Test (d)			P=0.232N
Fisher Exact Test (d)			P=0.233N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	1/49 (2%)	(f) 0/49 (0%)	5/50 (10%)
Adjusted Rates (b)	2.7%		11.1%
Terminal Rates (c)	1/37 (3%)		5/45 (11%)
Week of First Observation	104		104
Life Table Test (d)			P=0.153
Incidental Tumor Test (d)			P=0.153
Fisher Exact Test (d)			P=0.107
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	9/49 (18%)	(f) 5/49 (10%)	10/50 (20%)
Adjusted Rates (b)	22.7%		21.7%
Terminal Rates (c)	7/37 (19%)		9/45 (20%)
Week of First Observation	75		99
Life Table Test (d)			P=0.525N
Incidental Tumor Test (d)			P=0.574
Fisher Exact Test (d)			P=0.520
Liver: Hepatocellular Adenoma			
Overall Rates (a)	3/49 (6%)	(e) 1/10 (10%)	2/49 (4%)
Adjusted Rates (b)	8.1%		4.4%
Terminal Rates (c)	3/37 (8%)		2/45 (4%)
Week of First Observation	104		104
Life Table Test (d)			P=0.411N
Incidental Tumor Test (d)			P=0.411N
Fisher Exact Test (d)			P=0.500N

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	Control	600 ppm	1,200 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	4/49 (8%)	(e) 3/10 (30%)	4/49 (8%)
Adjusted Rates (b)	10.8%		8.9%
Terminal Rates (c)	4/37 (11%)		4/45 (9%)
Week of First Observation	104		104
Life Table Test (d)			P=0.533N
Incidental Tumor Test (d)			P=0.533N
Fisher Exact Test (d)			P=0.643
Pituitary Gland: Adenoma			
Overall Rates (a)	3/43 (7%)	(e) 1/9 (11%)	0/44 (0%)
Adjusted Rates (b)	9.4%		0.0%
Terminal Rates (c)	3/32 (9%)		0/40 (0%)
Week of First Observation	104		
Life Table Test (d)			P=0.085N
Incidental Tumor Test (d)			P=0.085N
Fisher Exact Test (d)			P=0.116N
Mammary Gland: Adenocarcinoma			
Overall Rates (a)	3/49 (6%)	2/49 (4%)	0/50 (0%)
Adjusted Rates (b)	7.7%	4.8%	0.0%
Terminal Rates (c)	2/37 (5%)	2/42 (5%)	0/45 (0%)
Week of First Observation	98	104	
Life Table Tests (d)	P=0.060N	P=0.452N	P=0.094N
Incidental Tumor Tests (d)	P=0.090N	P=0.649N	P=0.126N
Cochran-Armitage Trend Test (d)	P=0.079N		
Fisher Exact Test (d)		P=0.500N	P=0.117N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	3/48 (6%)	1/43 (2%)	3/49 (6%)
Adjusted Rates (b)	8.1%	2.1%	6.7%
Terminal Rates (c)	3/37 (8%)	0/37 (0%)	3/45 (7%)
Week of First Observation	104	69	104
Life Table Tests (d)	P=0.515N	P=0.307N	P=0.570N
Incidental Tumor Tests (d)	P=0.550N	P=0.271N	P=0.570N
Cochran-Armitage Trend Test (d)	P=0.583N		
Fisher Exact Test (d)		P=0.351N	P=0.651N
Harderian Gland: Adenoma or Papillary Cystadenoma			
Overall Rates (a)	3/49 (6%)	0/49 (0%)	3/50 (6%)
Adjusted Rates (b)	8.1%	0.0%	6.7%
Terminal Rates (c)	3/37 (8%)	0/42 (0%)	3/45 (7%)
Week of First Observation	104		104
Life Table Tests (d)	P=0.523N	P=0.100N	P=0.570N
Incidental Tumor Tests (d)	P=0.523N	P=0.100N	P=0.570N
Cochran-Armitage Trend Test (d)	P=0.593N		
Fisher Exact Test (d)		P=0.121N	P=0.651N
Harderian Gland: Adenoma, Papillary Cystadenoma, or Adenocarcinoma			
Overall Rates (a)	3/49 (6%)	0/49 (0%)	4/50 (8%)
Adjusted Rates (b)	8.1%	0.0%	8.9%
Terminal Rates (c)	3/37 (8%)	0/42 (0%)	4/45 (9%)
Week of First Observation	104		104
Life Table Tests (d)	P=0.491	P=0.100N	P=0.606
Incidental Tumor Tests (d)	P=0.491	P=0.100N	P=0.606
Cochran-Armitage Trend Test (d)	P=0.415		
Fisher Exact Test (d)		P=0.121N	P=0.511

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. N indicates a negative trend or lower incidence in a dosed group.
(e) All animals were examined grossly at the site and lesions found were evaluated microscopically. The incidence listed represents the number of animals with lesions diagnosed as tumors divided by the number of animals with gross lesions.
(f) Only 10 livers, 12 spleens, and 7 lymph nodes were examined.

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	49	50
INTEGUMENTARY SYSTEM			
*Skin	(49)	(49)	(50)
Ulcer, NOS	2 (4%)		
*Subcutaneous tissue	(49)	(49)	(50)
Fibrosis	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(48)	(10)	(50)
Inflammation, acute focal	2 (4%)		
Hyperplasia, epithelial	2 (4%)	1 (10%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(48)	(6)	(50)
Myelofibrosis	18 (38%)		12 (24%)
Hyperplasia, granulocytic	3 (6%)		1 (2%)
#Spleen	(48)	(12)	(49)
Angiectasis	1 (2%)		2 (4%)
#Splenic follicles	(48)	(12)	(49)
Depletion, lymphoid		1 (8%)	
#Splenic red pulp	(48)	(12)	(49)
Hematopoiesis	11 (23%)	7 (58%)	4 (8%)
#Lymph node	(48)	(7)	(47)
Abscess, chronic	1 (2%)		
#Mediastinal lymph node	(48)	(7)	(47)
Abscess, NOS	1 (2%)		1 (2%)
#Lumbar lymph node	(48)	(7)	(47)
Hemorrhagic cyst	1 (2%)		
Plasmacytosis			1 (2%)
#Mesenteric lymph node	(48)	(7)	(47)
Congestion, NOS	1 (2%)		
Hemorrhagic cyst	1 (2%)		
#Renal lymph node	(48)	(7)	(47)
Histiocytosis	1 (2%)		
Hematopoiesis	1 (2%)		
#Liver	(49)	(10)	(49)
Hematopoiesis	20 (41%)		17 (35%)
#Urinary bladder	(48)	(6)	(49)
Hyperplasia, lymphoid			1 (2%)
#Adrenal cortex	(49)	(6)	(50)
Hematopoiesis	1 (2%)		
#Adrenal medulla	(49)	(6)	(50)
Hematopoiesis	1 (2%)		
#Thymus	(38)	(4)	(45)
Depletion, lymphoid	3 (8%)	1 (25%)	
#Thymic lymphocytes	(38)	(4)	(45)
Necrosis, focal	1 (3%)		
CIRCULATORY SYSTEM			
#Lung	(48)	(10)	(50)
Thrombus, fibrin	1 (2%)		
#Heart	(49)	(7)	(50)
Endocarditis, bacterial	1 (2%)		
Inflammation, acute/chronic	1 (2%)		

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM (Continued)			
#Heart/atrium	(49)	(7)	(50)
Inflammation, chronic focal	1 (2%)		
#Myocardium	(49)	(7)	(50)
Degeneration, NOS	2 (4%)		
#Anterior pituitary	(43)	(9)	(44)
Thrombus, organized		1 (11%)	
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(7)	(49)
Atrophy, diffuse		1 (14%)	
Hyperplasia, focal			1 (2%)
#Liver	(49)	(10)	(49)
Congestion, NOS			1 (2%)
Hemorrhage, chronic	1 (2%)		
Inflammation, acute focal	4 (8%)		
Inflammation, acute/chronic	2 (4%)		
Necrosis, focal	3 (6%)		
Focal cellular change	1 (2%)		
#Pancreas	(47)	(10)	(48)
Dilatation/ducts	2 (4%)	4 (40%)	2 (4%)
Inflammation, acute/chronic		1 (10%)	
Inflammation, chronic focal			1 (2%)
Angiectasis	1 (2%)		
#Pancreatic acinus	(47)	(10)	(48)
Cytoplasmic change, NOS		1 (10%)	
Atrophy, focal		4 (40%)	
#Glandular stomach	(48)	(6)	(48)
Inflammation, acute focal	1 (2%)		
URINARY SYSTEM			
#Kidney	(49)	(8)	(49)
Glomerulonephritis, acute	1 (2%)		
Glomerulonephritis, chronic	5 (10%)		1 (2%)
Nephropathy	1 (2%)		4 (8%)
#Kidney/capsule	(49)	(8)	(49)
Inflammation, active chronic		1 (13%)	
#Kidney/tubule	(49)	(8)	(49)
Cytoplasmic aggregate, NOS		1 (13%)	
Regeneration, NOS	8 (16%)		12 (24%)
#Kidney/pelvis	(49)	(8)	(49)
Inflammation, suppurative	1 (2%)		
#Urinary bladder	(48)	(6)	(49)
Inflammation, acute focal	1 (2%)		
Inflammation, chronic focal	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary intermedia	(43)	(9)	(44)
Cytoplasmic vacuolization	1 (2%)		
Hyperplasia, focal			1 (2%)
#Anterior pituitary	(43)	(9)	(44)
Congestion, NOS	1 (2%)		
Hyperplasia, focal	2 (5%)	1 (11%)	4 (9%)
#Adrenal/capsule	(49)	(6)	(50)
Ectopia			2 (4%)
Hyperplasia, focal	44 (90%)	3 (50%)	47 (94%)
#Adrenal cortex	(49)	(6)	(50)
Cyst, NOS			1 (2%)
Focal cellular change	1 (2%)		
Hyperplasia, focal	2 (4%)		2 (4%)

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM (Continued)			
#Adrenal medulla	(49)	(6)	(50)
Hyperplasia, focal	1 (2%)		
#Thyroid	(48)	(6)	(49)
Follicular cyst, NOS	2 (4%)		3 (6%)
Inflammation, chronic focal	2 (4%)		
Hyperplasia, follicular cell	2 (4%)		1 (2%)
REPRODUCTIVE SYSTEM			
*Mammary gland	(49)	(49)	(50)
Inflammation, chronic focal	1 (2%)		
Hyperplasia, epithelial	1 (2%)		
#Uterus	(48)	(43)	(49)
Dilatation, NOS		2 (5%)	
Hemorrhage, chronic	1 (2%)		
Inflammation, acute focal	2 (4%)		2 (4%)
Inflammation, acute/chronic	2 (4%)	1 (2%)	2 (4%)
Angiectasis	1 (2%)		1 (2%)
#Endometrial gland	(48)	(43)	(49)
Hyperplasia, cystic	39 (81%)	38 (88%)	44 (90%)
#Ovary	(48)	(17)	(50)
Follicular cyst, NOS	15 (31%)	8 (47%)	13 (26%)
Parovarian cyst	4 (8%)	2 (12%)	4 (8%)
Inflammation, acute/chronic	2 (4%)		
Inflammation, chronic focal	2 (4%)		
Abscess, chronic	7 (15%)	3 (18%)	6 (12%)
Angiectasis	1 (2%)		
NERVOUS SYSTEM			
#Brain	(49)	(7)	(50)
Atrophy, pressure	1 (2%)		
SPECIAL SENSE ORGANS			
*Harderian gland	(49)	(49)	(50)
Hyperplasia, epithelial	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*Skeletal muscle	(49)	(49)	(50)
Inflammation, chronic focal	1 (2%)		
BODY CAVITIES			
*Mediastinum	(49)	(49)	(50)
Inflammation, acute focal	2 (4%)		1 (2%)
*Peritoneum	(49)	(49)	(50)
Inflammation, acute suppurative	2 (4%)	1 (2%)	
Inflammation, acute/chronic	3 (6%)		4 (8%)
Inflammation, chronic focal	1 (2%)		

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*Multiple organs	(49)	(49)	(50)
Inflammation, acute suppurative		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
Animal missing/no necropsy	1	1	
Auto/necropsy/histo perf		1	

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

APPENDIX E

GENETIC TOXICOLOGY OF ROTENONE

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TABLE E1. MUTAGENICITY OF ROTENONE IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	145 ± 13.5	159 ± 7.5	179 ± 10.1	134 ± 1.9	165 ± 8.4	127 ± 6.7
	100	139 ± 1.5	116 ± 9.7	175 ± 1.3	100 ± 1.7	140 ± 13.0	120 ± 5.2
	333	134 ± 17.0	122 ± 2.8	156 ± 1.5	101 ± 3.8	149 ± 9.4	123 ± 4.1
	1,000	146 ± 17.8	(c) 110 ± 15.8	181 ± 11.1	114 ± 10.6	135 ± 9.2	128 ± 2.0
	3,333	(c) 128 ± 8.2	(d) 118 ± 6.7	(c) 169 ± 5.0	(c) 115 ± 5.3	(c) 157 ± 11.1	(c) 131 ± 5.3
	10,000	(c) 119 ± 5.2	(d) 124 ± 6.9	(c) 150 ± 12.4	(c) 122 ± 1.2	(c) 160 ± 16.8	(c) 130 ± 5.8
	Trial Summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (e)	301 ± 3.1	416 ± 16.2	1,405 ± 54.5	1,785 ± 44.9	650 ± 26.8	712 ± 22.3
TA1535	0	25 ± 2.9	41 ± 2.1	35 ± 3.5	30 ± 2.8	34 ± 2.5	41 ± 1.8
	100	22 ± 2.5	34 ± 1.3	27 ± 1.7	35 ± 3.4	45 ± 3.8	25 ± 1.0
	333	25 ± 3.8	34 ± 2.9	24 ± 2.7	33 ± 3.1	27 ± 5.6	35 ± 1.5
	1,000	29 ± 7.5	(c) 27 ± 1.5	27 ± 1.3	37 ± 3.6	38 ± 3.2	26 ± 2.6
	3,333	(c) 28 ± 1.7	(c) 29 ± 4.3	(c) 23 ± 5.2	(c) 35 ± 4.9	(c) 32 ± 3.0	(c) 23 ± 3.8
	10,000	(c) 25 ± 2.6	(c) 25 ± 1.7	(c) 19 ± 0.3	(c) 25 ± 1.3	(c) 32 ± 3.7	(c) 19 ± 2.1
	Trial Summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (e)	290 ± 6.5	536 ± 4.4	361 ± 14.7	486 ± 4.3	167 ± 7.9	273 ± 6.9
TA1537	0	9 ± 2.3	10 ± 1.9	6 ± 1.8	8 ± 2.1	3 ± 1.2	10 ± 2.8
	100	10 ± 1.8	5 ± 1.8	5 ± 1.7	7 ± 1.2	10 ± 1.8	11 ± 1.5
	333	5 ± 0.3	10 ± 1.2	8 ± 1.9	5 ± 1.5	11 ± 2.7	9 ± 2.0
	1,000	9 ± 3.5	(c) 7 ± 1.5	13 ± 1.5	4 ± 0.0	14 ± 0.9	6 ± 0.0
	3,333	(c) 6 ± 0.9	(c) 4 ± 0.6	(c) 6 ± 2.0	(c) 7 ± 1.0	(c) 6 ± 0.9	(c) 6 ± 0.7
	10,000	(c) 9 ± 1.8	(c) 8 ± 2.0	(c) 7 ± 0.7	(c) 7 ± 1.0	(c) 9 ± 2.7	(c) 6 ± 1.0
	Trial Summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (e)	226 ± 29.6	667 ± 30.4	546 ± 9.9	537 ± 19.5	184 ± 20.9	178 ± 5.8
TA98	0	28 ± 1.2	28 ± 1.5	40 ± 1.8	40 ± 4.3	37 ± 4.1	38 ± 3.8
	100	23 ± 3.5	24 ± 5.4	34 ± 3.6	34 ± 3.7	37 ± 7.2	38 ± 3.5
	333	17 ± 0.3	25 ± 4.1	36 ± 2.4	31 ± 0.7	47 ± 5.2	31 ± 1.0
	1,000	19 ± 0.9	(c) 25 ± 2.5	38 ± 7.9	29 ± 1.0	39 ± 7.6	27 ± 3.8
	3,333	(c) 20 ± 4.6	(c) 24 ± 2.0	(c) 40 ± 0.6	(c) 29 ± 2.3	(c) 29 ± 5.5	(c) 36 ± 5.4
	10,000	(c) 20 ± 1.2	(c) 16 ± 2.5	(c) 40 ± 3.4	(c) 30 ± 1.7	(c) 32 ± 6.2	(c) 32 ± 3.2
	Trial Summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (e)	693 ± 35.3	852 ± 8.2	1,211 ± 47.4	1,738 ± 38.5	456 ± 22.4	560 ± 28.4

(a) Study performed at SRI International. The detailed protocol is presented in Haworth et al. (1983). Cells and study compound or solvent (95% ethanol) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. The high dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Precipitate on plate
(d) Slight toxicity
(e) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-o-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE E2. MUTAGENICITY OF ROTENONE IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

Compound	Concentration (µg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutant Count	Mutant Fraction (c)
-S9					
Trial 1					
Acetone		77.7 ± 9.4	100.0 ± 5.5	136 ± 10.5	59 ± 3.5
Rotenone	0.5	60.0 ± 5.0	21.0 ± 1.0	369 ± 30.5	(d) 208 ± 33.5
	1.0	55.5 ± 2.5	14.5 ± 0.5	530 ± 107.0	(d) 322 ± 80.5
	2.0	40.0 ± 9.0	6.5 ± 0.5	1,349 ± 569.5	(d) 1,310 ± 794.0
	4.0	15.0 ± 2.0	2.5 ± 0.5	1,369 ± 27.5	(d) 3,142 ± 510.5
	8.0	Lethal	--	--	--
Methyl methanesulfonate	15.0	38.0 ± 0.0	31.5 ± 0.5	268 ± 5.5	(d) 236 ± 4.5
Trial 2					
Acetone		77.8 ± 4.6	100.0 ± 3.9	165 ± 19.5	71 ± 6.7
Rotenone	0.25	64.0 ± 3.0	25.0 ± 1.0	222 ± 13.5	(d) 116 ± 1.0
	0.5	58.0 ± 8.0	32.5 ± 4.5	200 ± 18.0	(d) 119 ± 27.0
	1.0	65.0 ± 9.0	20.0 ± 3.0	451 ± 40.5	(d) 238 ± 54.0
	2.0	54.0 ± 4.0	17.0 ± 1.0	427 ± 15.5	(d) 268 ± 30.0
	4.0	Lethal	--	--	--
Methyl methanesulfonate	15.0	35.0 ± 0.0	28.0 ± 3.0	200 ± 41.5	(d) 191 ± 40.5

(a) Study performed at Inveresk Research International. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. All doses were tested in triplicate; the average of three tests is presented in the table. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

(b) Mean ± standard error of replicate tests for approximately 3×10^6 cells each. All data are evaluated statistically for both trend and peak response. Both responses must be significantly positive ($P<0.05$) for a chemical to be considered mutagenic. If only one of these responses is significant, the call is "questionable"; the absence of both trend and peak response results in a "negative" call.

(c) Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.

(d) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY ROTENONE (a)

	Dose (µg/ml)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hours in BrdU (b)	Relative SCEs/Cell (percent) (c)
−S9 (d)								
Trial No. 1--Summary: Negative								
Acetone		50	1,008	576	0.57	11.5	34.5	
Rotenone	0.001	50	1,003	585	0.58	11.7	34.5	101.7
	0.003	50	1,007	666	0.66	13.3	34.5	115.7
	0.004	0					34.5	
	0.008	0					34.5	
Mitomycin C	0.001	50	1,002	690	0.69	13.8	26.0	120.0
	0.010	5	104	193	1.86	38.6	25.0	335.7
+S9 (e)								
Trial No. 1--Summary: Positive								
Acetone		50	1,020	386	0.38	7.7	26.0	
Rotenone	0.2	50	1,017	450	0.44	9.0	34.0	116.9
	0.6	50	1,037	531	0.51	10.6	34.0	137.7
	2	9	188	97	0.52	10.8	34.0	140.3
	6	15	307	145	0.47	9.7	34.0	126.0
	20	0					34.0	
Cyclophosphamide	0.4	50	1,027	604	0.59	12.1	26.0	157.1
	2	5	105	187	1.78	37.4	26.0	485.7
Trial No. 2--Summary: Negative								
Acetone		50	1,017	602	0.59	12.0	26.0	
Rotenone	6	50	1,012	590	0.58	11.8	34.5	98.3
	10.1	50	1,010	611	0.60	12.2	34.5	101.7
	15.2	50	1,002	598	0.60	12.0	34.5	100.0
	20	0					34.5	
Cyclophosphamide	0.4	50	1,006	831	0.83	16.6	26.0	138.3
	2	5	102	177	1.74	35.4	26.0	295.0

(a) Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (acetone) as described in (d) or (e) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

(b) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.

(c) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to solvent (acetone)

(d) In the absence of S9, cells were incubated with study compound or solvent (acetone) for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.

(e) In the presence of S9, cells were incubated with study compound or solvent (acetone) for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE E4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY ROTENONE (a)

Trial 1					Trial 2				
Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9--Harvest time: 21.5 hours (b,c)					**−S9--Harvest time: 20.5 hours (b,c)**				
Acetone	100	1	0.01	1	Acetone	100	5	0.05	4
Rotenone					Rotenone				
10	100	2	0.02	2	25	100	0	0.00	0
25	100	11	0.11	10	75	100	3	0.03	3
50	100	3	0.03	2	100	100	2	0.02	2
Summary: Equivocal					Summary: Negative				
Mitomycin C					Mitomycin C				
0.040	100	22	0.22	14	0.05	25	48	1.92	72
0.063	25	20	0.80	32	0.08	10	27	2.70	80
+S9--Harvest time: 21.5 hours (c,d)									
Acetone	100	3	0.03	3					
Rotenone									
100	100	9	0.09	5					
150	100	7	0.07	6					
200	100	8	0.08	7					
250	0								
Summary: Negative									
Cyclophosphamide									
6.25	100	16	0.16	13					
12.50	50	28	0.56	26					

(a) Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as indicated in (b) or (d). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

(b) In the absence of S9, cells were incubated with study compound or solvent (acetone) for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.

(c) Because of significant chemical-induced cell cycle delay, incubation time before addition of colcemid was lengthened to provide sufficient metaphases at harvest.

(d) In the presence of S9, cells were incubated with study compound or solvent (acetone) for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

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APPENDIX F

SENTINEL ANIMAL PROGRAM

APPENDIX F. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen B6C3F$_1$ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests are performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus)	MHV (mouse hepatitis virus)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus)	*M. pul.* (*Mycoplasma pulmonis*) (tested at 24 months only)

II. Results

Three of 10 control rats tested positive for *Mycoplasma pulmonis* at 24 months. No positive results were obtained in mice. *M. pulmonis* infection-related lesions were not observed in the rats in this study. Further evaluation of the reagents used for detection of *M. pulmonis* by ELISA indicated that the reagents used may not be specific for detection of antibodies to *M. pulmonis*.

APPENDIX G

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF ROTENONE

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TABLE G1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	21	230	21	224	1.0	3.6	21	224	1.0	7.0
4	19	277	18	272	0.9	2.5	19	273	1.0	5.2
8	17	325	18	321	1.1	2.1	17	322	1.0	4.0
12	18	348	16	347	0.9	1.8	17	350	0.9	3.6
17	18	385	17	379	0.9	1.7	17	382	0.9	3.3
22	22	403	21	388	1.0	2.1	22	405	1.0	4.1
26	18	412	19	414	1.1	1.7	19	416	1.1	3.4
30	17	427	17	426	1.0	1.5	17	430	1.0	3.0
35	17	440	17	442	1.0	1.5	17	442	1.0	2.9
40	16	454	19	454	1.2	1.6	18	451	1.1	3.0
44	17	459	18	460	1.1	1.5	18	456	1.1	3.0
49	19	461	19	464	1.0	1.6	18	460	0.9	2.9
54	17	463	18	465	1.1	1.5	18	459	1.1	2.9
58	16	461	17	469	1.1	1.4	17	465	1.1	2.7
62	17	464	17	465	1.0	1.4	17	458	1.0	2.8
66	21	465	21	466	1.0	1.7	21	460	1.0	3.4
70	18	464	17	465	0.9	1.4	18	459	1.0	2.9
75	18	471	18	467	1.0	1.5	17	461	0.9	2.8
79	17	464	18	473	1.1	1.4	18	460	1.1	2.9
83	18	472	18	467	1.0	1.5	17	463	0.9	2.8
88	17	472	18	467	1.1	1.5	17	464	1.0	2.7
93	17	466	16	457	0.9	1.3	19	467	1.1	3.1
97	17	456	17	454	1.0	1.4	18	457	1.1	3.0
101	18	448	18	446	1.0	1.5	20	448	1.1	3.3
Mean	17.9	424	18.0	423	1.0	1.7	18.2	422	1.0	3.4
SD (d)	1.5		1.4		0.1	0.5	1.5		0.1	1.0
CV (e)	8.4		7.8		10.0	29.4	8.2		10.0	29.4

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of rotenone consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE G2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
1	13	153	13	154	1.0	3.2	13	157	1.0	6.2
4	12	172	12	171	1.0	2.7	12	172	1.0	5.2
8	12	189	11	187	0.9	2.2	11	187	0.9	4.4
12	12	196	10	195	0.8	1.9	11	194	0.9	4.3
17	11	209	10	207	0.9	1.8	10	206	0.9	3.6
22	11	219	11	218	1.0	1.9	10	216	0.9	3.5
26	13	223	12	220	0.9	2.1	12	217	0.9	4.1
30	12	230	11	226	0.9	1.8	12	226	1.0	4.0
35	12	236	12	234	1.0	1.9	12	232	1.0	3.9
40	12	250	11	245	0.9	1.7	12	244	1.0	3.7
44	13	261	11	256	0.8	1.6	11	254	0.8	3.2
49	13	271	12	264	0.9	1.7	12	259	0.9	3.5
54	12	280	11	275	0.9	1.5	11	270	0.9	3.1
58	12	291	11	284	0.9	1.5	11	277	0.9	3.0
62	13	293	12	286	0.9	1.6	12	276	0.9	3.3
66	16	305	15	292	0.9	2.0	15	282	0.9	4.0
70	13	309	12	295	0.9	1.5	13	282	1.0	3.5
75	13	326	13	312	1.0	1.6	13	297	1.0	3.3
79	12	331	11	318	0.9	1.3	12	304	1.0	3.0
83	13	337	12	325	0.9	1.4	12	313	0.9	2.9
88	12	341	11	324	0.9	1.3	12	322	1.0	2.8
93	12	339	12	331	1.0	1.4	13	331	1.1	2.9
97	13	341	13	325	1.0	1.5	12	332	0.9	2.7
101	13	350	14	330	1.1	1.6	13	333	1.0	2.9
Mean	12.5	269	11.8	261	0.9	1.8	12.0	258	1.0	3.6
SD (d)	1.0		1.2		0.1	0.4	1.1		0.1	0.8
CV (e)	8.0		10.2		11.1	22.2	9.2		10.0	22.2

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of rotenone consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE G3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

Week	Control		Low Dose				High Dose			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
5	5	28.8	5	28.5	1.0	105	6	26.2	1.2	275
8	5	29.6	5	28.9	1.0	104	5	28.0	1.0	214
12	6	31.8	5	31.7	0.8	95	5	30.7	0.8	195
15	6	32.0	6	31.6	1.0	114	7	29.7	1.2	283
20	7	32.3	8	31.8	1.1	151	8	30.2	1.1	318
24	5	34.9	5	34.1	1.0	88	6	32.1	1.2	224
29	5	37.0	6	34.1	1.2	106	6	32.8	1.2	220
33	7	37.8	6	35.6	0.9	101	6	34.0	0.9	212
37	6	39.1	6	35.8	1.0	101	6	34.4	1.0	209
42	6	38.2	6	36.3	1.0	99	6	34.4	1.0	209
47	6	40.0	6	37.3	1.0	97	6	35.4	1.0	203
51	6	40.7	6	38.0	1.0	95	7	35.7	1.2	235
56	6	41.1	6	38.1	1.0	94	6	35.2	1.0	205
60	6	41.0	7	37.7	1.2	111	6	35.2	1.0	205
64	5	41.9	6	37.5	1.2	96	6	35.7	1.2	202
69	6	42.4	7	37.5	1.2	112	7	35.2	1.2	239
73	8	42.4	8	36.5	1.0	132	8	34.6	1.0	277
77	8	44.0	8	38.1	1.0	126	8	35.8	1.0	268
81	6	42.7	7	37.3	1.2	113	7	35.1	1.2	239
87	7	41.3	7	37.0	1.0	114	8	35.6	1.1	270
91	8	42.6	8	36.7	1.0	131	8	34.7	1.0	277
94	6	41.1	6	36.4	1.0	99	7	35.2	1.2	239
99	10	37.9	10	35.7	1.0	168	10	33.9	1.0	354
Mean	6.3	38.3	6.5	35.3	1.0	111	6.7	33.5	1.1	242
SD (d)	1.2		1.2		0.1	19	1.2		0.1	41
CV (e)	19.0		18.5		10.0	17.1	17.9		9.1	16.9

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of rotenone consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE G4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF ROTENONE

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
5	4	21.4	5	20.7	1.3	145	5	20.8	1.3	288
8	4	22.6	4	22.0	1.0	109	6	21.6	1.5	333
12	5	24.4	5	23.6	1.0	127	6	23.4	1.2	308
15	6	25.7	6	24.0	1.0	150	6	23.6	1.0	305
20	8	27.6	8	25.4	1.0	189	8	24.7	1.0	389
24	5	30.0	5	27.0	1.0	111	5	27.0	1.0	222
29	5	30.7	6	27.8	1.2	129	5	26.6	1.0	226
33	5	31.4	6	27.6	1.2	130	6	26.3	1.2	274
37	5	32.9	6	28.9	1.2	125	5	27.4	1.0	219
42	5	33.2	6	29.4	1.2	122	6	27.9	1.2	258
47	5	35.6	6	30.0	1.2	120	6	28.5	1.2	253
51	5	36.5	6	31.8	1.2	113	5	29.7	1.0	202
56	5	37.6	5	31.6	1.0	95	5	29.4	1.0	204
60	5	37.3	5	32.0	1.0	94	6	29.7	1.2	242
64	5	38.7	5	32.8	1.0	91	5	30.7	1.0	195
69	5	39.2	6	32.1	1.2	112	6	29.7	1.2	242
73	6	41.1	7	32.1	1.2	131	7	28.6	1.2	294
77	6	41.6	7	34.4	1.2	122	7	31.0	1.2	271
81	5	41.1	6	33.6	1.2	107	6	31.0	1.2	232
87	5	43.5	8	35.4	1.6	136	7	32.4	1.4	259
91	6	44.5	7	35.4	1.2	119	7	32.0	1.2	263
94	5	44.2	6	35.6	1.2	101	7	32.7	1.4	257
99	9	44.6	10	36.1	1.1	166	10	33.1	1.1	363
Mean 5.4	35.0	6.1	30.0	1.1	124	6.2	28.2	1.2	265	
SD (d)	1.1		1.3		0.1	23	1.2		0.1	50
CV (e)	20.4		21.3		9.1	18.5	19.4		8.3	18.9

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Estimated milligrams of rotenone consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

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APPENDIX H

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Meal Diet: April 1981 to April 1983

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

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TABLE H4	CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION	154

TABLE H1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredient (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Brewer's dried yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NIH, 1978; NCI, 1976
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE H2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamin		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione activity
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Mineral		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE H3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Crude protein (percent by weight)	24.19 ± 1.07	22.4-26.3	25
Crude fat (percent by weight)	5.02 ± 0.47	4.2-6.0	25
Crude fiber (percent by weight)	3.37 ± 0.37	2.4-4.2	25
Ash (percent by weight)	6.54 ± 0.26	5.97-7.03	25
Essential Amino Acid (percent of total diet) (a)			
Arginine	1.300	1.21-1.38	3
Cystine	0.340	0.23-0.40	3
Glycine	1.137	1.06-1.20	3
Histidine	0.561	0.530-0.578	3
Isoleucine	0.899	0.881-0.934	3
Leucine	1.930	1.85-1.98	3
Lysine	1.243	1.20-1.30	3
Methionine	0.329	0.306-0.368	3
Phenylalanine	0.991	0.960-1.04	3
Threonine	0.851	0.827-0.886	3
Tryptophan	0.187	0.171-0.211	3
Tyrosine	0.647	0.566-0.769	3
Valine	1.090	1.05-1.12	3
Essential Fatty Acid (percent of total diet) (a)			
Linoleic	2.40	2.37-2.44	2
Linolenic	0.284	0.259-0.308	2
Vitamin (a)			
Vitamin A (IU/kg)	11,936 ± 2,547	8,900-22,000	25
Vitamin D (IU/kg)	5,220	4,140-6,300	2
α-Tocopherol (ppm)	39.1	31.1-44.0	3
Thiamine (ppm)	18.7 ± 3.20	14.0-26.0	(b) 24
Riboflavin (ppm)	7.3	6.1-8.1	3
Niacin (ppm)	82	65-97	3
Pantothenic acid (ppm)	30.2	23.0-30.5	3
Pyridoxine (ppm)	7.7	5.6-8.8	3
Folic acid (ppm)	2.5	1.8-3.4	3
Biotin (ppm)	0.27	0.21-0.32	3
Vitamin B_{12} (ppb)	21.2	10.6-38.0	3
Choline (ppm)	3,337	3,200-3,430	3
Mineral (a)			
Calcium (percent)	1.22 ± 0.10	1.10-1.45	25
Phosphorus (percent)	0.96 ± 0.05	0.84-1.10	25
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.581	0.479-0.635	3
Sodium (percent)	0.307	0.258-0.349	3
Magnesium (percent)	0.165	0.151-0.177	3
Sulfur (percent)	0.292	0.270-0.290	3
Iron (ppm)	420	409-431	3
Manganese (ppm)	87.7	81.7-95.5	3
Zinc (ppm)	52.1	46.1-56.0	3
Copper (ppm)	11.15	8.09-15.70	3
Iodine (ppm)	2.66	1.52-3.64	3
Chromium (ppm)	1.72	1.44-1.93	3
Cobalt (ppm)	0.64	0.49-0.78	3

(a) Two or three batches of feed analyzed for nutrients reported in this table were manufactured in 1983 and 1984.
(b) One batch (7/22/81) was not analyzed for thiamine.

TABLE H4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminant	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.45 ± 0.11	0.21-0.65	25
Cadmium (ppm) (a)	<0.1		25
Lead (ppm)	0.95 ± 0.78	0.27-2.93	25
Mercury (ppm) (a)	< 0.05		25
Selenium (ppm)	0.28 ± 0.06	0.16-0.40	25
Aflatoxins (ppb) (a,b)	<10	<5.0-<10.0	25
Nitrate nitrogen (ppm) (c)	9.85 ± 4.55	0.6-19.0	25
Nitrite nitrogen (ppm) (c)	1.92 ± 1.28	0.4-5.3	25
BHA (ppm) (d)	5.67 ± 5.07	1.5-20.0	25
BHT (ppm) (d)	3.35 ± 2.55	<1.0-13.0	25
Aerobic plate count (CFU/g)	121,420 ± 94,544	7,000-420,000	25
Coliform (MPN/g) (e)	965 ± 991	<3-2,400	25
E. coli (MPN/g) (e,f)	6.76 ± 7.06	<3-23	24
E. coli (MPN/g) (e,g)	12.64 ± 29.46	<3-150	25
Total nitrosamines (ppb) (h,i)	4.40 ± 3.16	<1.2-12.9	24
Total nitrosamines (ppb) (h, j)	8.29 ± 19.41	<1.2-100.3	25
N-Nitrosodimethylamine (ppb) (h,k)	3.05 ± 3.05	0.6-12.0	24
N-Nitrosodimethylamine (ppb) (h,l)	6.89 ± 19.42	0.6-99.0	25
N-Nitrosopyrrolidine (ppb)	1.20 ± 0.62	<0.3-2.4	25
Pesticide (ppm)			
α-BHC (a,m)	<0.01		25
β-BHC (a)	<0.02		25
γ-BHC-Lindane (a)	<0.01		25
δ-BHC (a)	<0.01		25
Heptachlor (a)	<0.01		25
Aldrin (a)	<0.01		25
Heptachlor epoxide (a)	<0.01		25
DDE (n)	<0.01	0.05 (7/14/81)	25
DDD (a)	<0.01		25
DDT (a)	<0.01		25
HCB (a)	<0.01		25
Mirex (a)	<0.01		25
Methoxychlor (o)	<0.05	0.13 (8/25/81); 0.6 (6/29/82)	25
Dieldrin (a)	<0.01		25
Endrin (a)	<0.01		25
Telodrin (a)	<0.01		25
Chlordane (a)	<0.05		25
Toxaphene (a)	<0.1		25
Estimated PCB's (a)	<0.2		25
Ronnel (a)	<0.01		25
Ethion (a)	<0.02		25
Trithion (a)	<0.05		25
Diazinon (a)	<0.1		25
Methyl parathion (a)	<0.02		25
Ethyl parathion (a)	<0.02		25
Malathion (p)	0.08 ± 0.05	<0.05-0.25	25
Endosulfan I (a)	<0.01		25
Endosulfan II (a)	<0.01		25
Endosulfan sulfate (a)	<0.03		25

TABLE H4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) All values were less than the detection limit, given in the table as the mean.
(b) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) MPN = most probable number
(f) Mean, standard deviation, and range exclude one value of 150 for batch produced on 8/26/82.
(g) Mean, standard deviation, and range include the high value given in footnote f.
(h) All values were corrected for percent recovery.
(i) Mean, standard deviation, and range exclude one value of 100.3 ppb for batch produced on 4/27/81.
(j) Mean, standard deviation, and range include the high value given in footnote i.
(k) Mean, standard deviation, and range exclude one value of 99 for batch produced on 4/27/81.
(l) Mean, standard deviation, and range include the high value listed in footnote k.
(m) BHC = hexachlorocyclohexane or benzene hexachloride
(n) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(o) Two observations were above the detection limit. The value and the date they were obtained are listed under the range.
(p) Ten batches contained more than 0.05 ppm.

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APPENDIX I

DATA AUDIT SUMMARY

APPENDIX I. DATA AUDIT SUMMARY

The experimental data, documents, pathology materials, and draft Technical Report for the 2-year toxicology and carcinogenesis studies of rotenone in rats and mice were audited for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations of the Food and Drug Administration (implemented by the NTP beginning on October 1, 1981). The laboratory experiments were conducted for the NTP by Battelle Columbus Laboratories, Columbus, Ohio, under a subcontract with Tracor Jitco, Inc., until 1982 and then under contract with the NIEHS. Animal exposures to rotenone began in June 1981 and ended in June 1983. The retrospective audit was conducted at the NTP Archives in January and May 1986 by Argus Research Laboratories. The following individuals were involved in the audit: Paul A. Wennerberg, D.V.M., M.S. (Principal Investigator); Lynn E. Blalock, M.S.; Betty L. Brandau, Ph.D.; Patricia D. Hall; Bonnie Jo Johnson; Sharon H. Srebro, B.S.; Stephanie M. Taulbee; and Kathleen M. Walsh, D.V.M., D.A.C.V.P.

The full report of the audit is on file at the NIEHS. The audit followed NTP standard operating procedures and included a review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All chemistry records.
(3) Body weight and clinical observation data for a random 10% sample of the study animals.
(4) Twenty-five percent of the feed consumption data for each group of animals.
(5) All inlife records concerning environmental conditions, masses, mortality, and animal identification.
(6) All postmortem records for individual animals concerning identification, disposition codes, condition codes, and correlation between gross observations and microscopic diagnoses.
(7) Wet tissues from a random 10% sample of the study animals to verify animal identification and to examine for untrimmed potential lesions.
(8) Slides and blocks of tissues from all control and high dose animals to examine for proper match and inventory.
(9) Tabulated pathology diagnoses for a random 10% of study animals to verify computer data entry.

The audit showed that the records were complete with the exception of records for the disposition of extra animals at the time of study start and chemistry notebooks for the first month of the study. The audit findings indicated that the inlife and chemistry portions of the studies were conducted and documented with no incidents that would influence the interpretation of study results. The examination of approximately 4,000 individual residual wet tissues from 95 animals indicated that animals were identified adequately. A total of 10 untrimmed potential lesions were found, and 10 gross observations had no corresponding microscopic diagnosis.

The audit findings were reviewed and interpreted by NTP staff. The untrimmed potential lesions and gross observations not microscopically correlated were few in number and were distributed among different organs and study groups; thus, no additional diagnoses were performed, since they would not have affected the interpretations of the studies. In conclusion, the documents and materials at the NTP Archives support the results presented in this Technical Report.

•U.S. GOVERNMENT PRINTING OFFICE:1998-201-779:51197

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PUBLISHED AS OF OCTOBER 1987

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	Dibromochloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane (Inhalation)
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butylbenzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-*p*-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	D-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos
250	Benzyl Acetate
251	Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
267	Propylene Oxide
269	Telone II®
271	HC Blue No. 1
272	Propylene
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
281	H.C. Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethy)phosphonium Sulfate and Tetrakis(hydroxymethy)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	o-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
324	Boric Acid
325	Pentachloronitrobenzene
327	Xylenes (Mixed)

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NIH Publication No. 88-2576
January 1988